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SNAKE VENOMS

III. Immunity[1]

CHARLES H. KELLAWAY, M.C., M.D., M.S. (Melb.), F.R.C.P. (Lond.)

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In addition to yielding the only efficient weapon for combating the ill effects of snake bite, studies in immunity to snake venoms have thrown light upon the specificity of immune reactions, upon the vexed question of cellular immunity and upon natural immunity, of which the immunity of serpents to their own venoms furnishes a conspicuous example.

In 1887 Sewell, of Ann Arbor, first showed that it was possible by repeated inoculation with small doses of the venom of the Massasauga (*Sistrurus catenatus*) to immunise pigeons against a lethal dose of the venom. At the present day, in numerous serum institutes in many countries antivenenes are prepared for the treatment of the bites of venomous snakes.

It early became apparent that antivenene prepared against the venom of an individual snake was highly specific in its action. This was demonstrated by the early researches of Martin (1897), Lamb (1904–5), Flexner and Noguchi (1904) and Tidswell (1906). The more recent studies of Arthus (1911), of Houssay and his colleagues (1923), of Brazil (1914) and of Vellard (1930), have only modified our viewpoint to the extent of showing that some slight degree of protection may be afforded by monovalent antivenenes against the venoms of species closely allied to those used for their preparation.

[1] Lecture III of the twelfth course of lectures under The Charles E. Dohme Memorial Lectureship of The Johns Hopkins University School of Medicine. Delivered November 7, 1936. Lecture I: "Snake Venoms—Their Constitution and Therapeutic Applications" appears on pp. 1–17. Lecture II: "The Peripheral Action of Snake Venoms" appears on pp. 18–39 of this volume (ZX) of the Bulletin.

The degree of specificity exhibited is well illustrated by the behaviour of an antivenene prepared by Dr. F. G. Morgan against the venom of the species of tiger snake (*Notechis scutatus*) which is common on the mainland of Australia. This, in a dose of 1.0 cc., was capable of neutralising about 80 guinea-pig lethal doses of the venom used in its preparation, about 20 lethal doses of that of a melanotic variant of the same species from Chappell Island and about 7 lethal doses of that of the closely allied copperhead, but had no significant protective effect against the venoms of the death adder, black snake or brown snake.

Miss Williams and I (1931) have studied the serological and blood relationships of these snakes by precipitin and complement fixation tests of their sera and by their haemagglutination reactions, using antisera prepared in rabbits. These tests revealed extremely close antigenic relationships among these Australian venomous species and particularly between the tiger snake and the copperhead. Precipitin tests showed striking cross reactions. Complement fixation was more specific and the results accurately arranged the genera in their zoological order—*Demansia*, *Pseudechis*, *Denisonia*, *Notechis* and *Acanthophis*, though they did not enable us to allot species to their genera.

Another factor enters to disturb the parallelism between the blood relationships of snakes and the antigenic relationships of their venoms, namely, the variations in the toxic constitution of the latter.

The venom of the brown snake (genus *Demansia*) is powerfully curarizing and coagulant and less strikingly haemolytic, with a tendency to cause haemorrhages; the venom of the black snake (genus *Pseudechis*) is powerfully haemolytic, haemorrhagic and coagulant but only feebly curarizing; that of the copperhead (genus *Denisonia*) is strongly curarizing and haemolytic and feebly anticoagulant; that of the tiger snake (genus *Notechis*) strongly coagulant, curarizing, and weakly haemolytic, and that of the death adder (genus *Acanthophis*) is strongly curarizing, moderately haemorrhagic and feebly haemolytic and anticoagulant.

These differences in toxic constitution probably account for the total absence of passive protection afforded by potent tiger snake antivenene against the venoms of the closely allied black snake, brown snake and death adder.

The study of the specificity of active immunity to these venoms

further illustrates the influence of these two factors—the serological relationship of the reptiles and the toxic constitution of their venoms—in determining reactions to the venoms of allied species, which are less strictly specific than those observed in passive protection by antivenenes.

Guinea-pigs actively immune to 14 lethal doses of tiger-snake venom were protected against 12 lethal doses of copperhead venom. Only slight and uncertain protection was observed against $1\frac{1}{4}$ lethal doses of death-adder venom and none at all against the venoms of the common brown and black snake.

Guinea-pigs actively immunised against 9 certainly lethal doses of copperhead venom were protected against 8 to 12 lethal doses of tiger-snake venom. Irregular protection was observed against a single lethal dose of death-adder venom but none against the venoms of the black snake or of the giant brown snake. A single animal survived the injection of a little more than a lethal dose of brown-snake venom.

Neither of these groups of animals showed any protection at all against the venom of the Indian Cobra (*Naia naia*), a powerfully curarizing and haemolytic poison with anticoagulant action.

Guinea-pigs immunised against 2 certainly lethal doses of death-adder venom were protected against 8 and 10 lethal doses of tiger-snake and copperhead venom, respectively. These animals had acquired a high immunity to the curarizing constituents of these venoms. They were difficult to immunise because of the haemorrhagic property of death-adder venom, and since the early immunising injections were made with venom detoxicated by formalin, which preferentially destroys haemorrhagin, they may have reached a high titre against the curarizing activity of this venom.

A similar phenomenon was observed with serum from horses immunised with unaltered death-adder venom. Such an immune serum of which 1 cc. neutralised only 4 guinea-pig lethal doses of death-adder venom was found to neutralise 12 certainly lethal doses of tiger-snake venom and 2 lethal doses of copperhead venom per cubic centimeter.

Guinea-pigs immunised against 2 certainly lethal doses of cobra venom showed only a feeble and irregular protection against the venoms of the Australian copperhead, death adder and tiger snake—one or two animals surviving single lethal doses of these venoms.

The most striking feature in these experiments was the protection

afforded against the venoms of the tiger snake and copperhead by immunisation with death-adder venom, though immunisation with tiger-snake and copperhead venoms yielded no appreciable protection against the venom of the death adder. This is probably to be explained by the presence of two antigenic substances in death-adder venom, one associated with a "haemorrhagin" and the other with a curarizing constituent antigenically similar to that in tiger-snake and copperhead venoms.

Vellard (1930), in experiments on the specificity of passive immunity to viperine venoms, observed a somewhat similar phenomenon. His "anti-bothropique" and "anti-jararaca" sera gave higher protection in lethal doses, though not in milligrammes of venom, against the venom of *Crotalus terrificus* than against the *Bothrops* venoms used in their preparation.

Two factors might conceivably play a part in causing the less strict specificity observed in animals actively immunised with snake venoms than in passive protection experiments with immune sera. The closely allied venoms of similar toxic constitution might be able to function as specific secondary stimuli, or there might be an acquired general tissue resistance (cellular immunity).

If the venoms are nearly enough allied antigenically to act as specific secondary stimuli, time relationships must to a large extent determine the protective value of this reaction in the actively immune animal. Can antibody be produced by a secondary stimulus sufficiently rapid to account for any significant additional protection? Dean and Webb (1928) have shown that the secondary precipitin response to horse serum in an immune rabbit takes seven days to become maximal and during the first two days no appreciable response is to be observed. Burnet[2] has shown that in rabbits immunised against staphylococcal toxin an injection of toxoid causes an immediate fall in the titre of circulating antibody. At the end of the second day the titre of antibody commences to rise, reaching a peak about the fifth day.

Now the death times following the injection of snake venom depend on the size of the dose (Acton and Knowles (1913), Glenny (1925)) and also upon the nature of the venom. In the case of the venoms of

[2] Unpublished observations.

the tiger snake and copperhead, which are strongly curarizing in action, death, both in rabbits and guinea-pigs, when only a single certainly lethal dose is given subcutaneously, takes place on the second, third or fourth days. With black-snake venom which is powerfully haemorrhagic, death, in both the rabbit and guinea-pig, usually occurs in less than eighteen hours. With death-adder venom, death is also rapid, nearly all the animals dying during the first day. It is evident then that in an animal highly immune to one venom the action of a closely allied venom as a secondary specific stimulus is more likely to be efficient as a protective mechanism following injection of the venoms of the tiger snake and copperhead, than of those of the black snake and death adder.

In the rabbit, it is possible to titrate the humoral immunity at the time of testing with other venoms, and to ascertain whether closely allied venoms can act as secondary stimuli.

Miss Williams and I carried out experiments of this kind on rabbits immunised to high titre with tiger-snake, copperhead and death-adder venoms, respectively. We found that in animals immunised against one venom, the immunity to the two closely related venoms was greater than was to be predicted from the titre of their sera just before testing. There were two exceptions to this general statement. Rabbits immunised with copperhead or with death-adder venom and tested with tiger-snake venom did not show as great a protection as would have been predicted. They frequently died from intravascular coagulation following the subcutaneous injection of the large doses of tiger-snake venom necessary for testing.

We were able to demonstrate that the closely allied venoms were sufficiently similar antigenically to act in these animals as specific secondary stimuli. A rabbit immunised with tiger-snake venom, the titre of whose serum to death-adder venom was 0.017–0.02 mg. per cubic centimeter, and to tiger-snake venom 0.15 mg., received 3 lethal doses of death-adder venom. Six days later the titre to death-adder venom was 0.05 mg. and that to tiger-snake venom 0.22 mg. per cubic centimeter.

In another tiger-snake immune rabbit in which a long time had elapsed since the last immunising injection, the titre before the injection of $1\frac{3}{4}$ lethal doses of copperhead venom was 0.02 mg. of tiger-snake

and 0.01 mg. of copperhead venom per cubic centimeter. On the fourth day after the injection the titre had arisen to 0.11 mg. of tiger-snake and to more than 0.06 mg. of copperhead and by the eighth day to 0.5 mg. of tiger-snake and 0.45 mg. of copperhead venom per cubic centimeter.

These results make it certain that the closely allied venoms act as specific secondary stimuli, and would serve to explain the relatively high protection observed in these actively immunised animals to venoms other than that used for immunisation, if sufficient time were afforded for the production of antibody. Our observations unfortunately are not conclusive upon this last point, but by analogy with Burnet's results in animals immunised against staphylococcal toxin it is possible, though rather unlikely, that the action of closely allied venoms as secondary stimuli may play a part in determining protection in these actively immune animals against such closely allied venoms as kill slowly, when present in barely lethal dosage.

CELLULAR IMMUNITY

There is no valid evidence that the tissues of actively immune animals, independently of the production of humoral antibody, can acquire immunity to antigenic toxic substances. Gunn and Heathcote (1920) found that the perfused heart and intestine from a single actively immune rabbit were more resistant to cobra venom than the perfused tissues of normal rabbits. Manwaring and Williams (1923) were unable to confirm these results. Miss Williams and I (1931) tested perfused isolated auricles from rabbits highly immune to cobra venom and found them more resistant than those from normal animals. Cobra venom in a concentration of 1:140,000 caused cessation of beat in 4 preparations from normal rabbits in 6, 7, 10, and $12\frac{1}{2}$ minutes, but of 4 preparations from immune animals two ceased to beat in 29 and 65 minutes and two were still feebly beating after 45 and 75 minutes, respectively. We found, however, that small amounts of antitoxic antibody added to the bath in which normal auricles were suspended had a disproportionately large protective effect. Sufficient immune serum to neutralise 0.1 mg. of venom added to a bath of 140 cc. capacity prior to the administration of 1.0 mg. of venom gave cessation times of $12\frac{1}{2}$, 31 and 100 minutes for three normal auricles,

and half this concentration of antibody gave cessation times in three further experiments of 9, 20 and 40 minutes. Markowitz, Essex and Mann (1931) found that perfused hearts from rabbits immune to *Crotalus* venom behaved exactly like those from normal rabbits, but also observed the highly protective action of small amounts of rabbit immune plasma.

Miss Williams and I concluded that our positive results could not be regarded as evidence that the cardiac muscle could acquire antitoxic tissue immunity but were probably due to the presence of humoral antibody which had not been removed by the preliminary perfusion of 500–600 cc. of Ringer's solution through the coronary circulation.

Burnet, Miss Williams and I (1932) used staphylococcal toxin for further study of this question. This toxin, when injected intravenously, in rabbits causes intense pulmonary vasoconstriction, constriction of the coronary vessels and rapid failure of the heart (Kellaway, Burnet and Williams, 1930). We tested the possibility of the acquisition of tissue immunity on the perfused lungs of immune rabbits and upon the heart perfused through the coronary circulation. In neither was there any evidence of tissue immunity in highly immune animals. Preparations from immune rabbits showed instead a slight diminution of resistance, which was probably due to anaphylactic sensitiveness to the toxin itself or to some antigenic component of the crude toxin.

There is a fundamental difference between these experiments and those with cobra venom. Here we were concerned with the reaction of cells which form part of the blood-vessel walls and which could more readily be washed free from immune bodies by perfusion. Believing that the difference in the results of the two sets of experiments must depend on the accumulation of antibody in the tissue spaces from which it could not readily be removed by perfusion, we sought evidence of such accumulation in immune animals by first thoroughly perfusing the whole animal or organ with 2 to 3 litres of Ringer, and then inducing an oedematous exudate, the antibody content of which was accurately titrated.

In experiments in which the whole animal was perfused with Ringer's solution through the jugular vein, peritoneal exudate and lung oedema

fluid were collected and leakage from the circulation was controlled by the intravenous injection, before the perfusion started, of a small amount of high titre agglutinating serum. Antitoxin was present in the peritoneal exudate to an amount much in excess of agglutinating antibody and the lung oedema fluid contained relatively large amounts of antitoxin but no detectable agglutinin. The oedema fluid obtained from normal rabbits by the same technique contained no demonstrable antibody.

When the lungs alone were perfused, we were able to rid the perfusing fluid of antitoxin and passively added agglutinin and then to obtain from the trachea an oedematous exudate which contained antitoxin equivalent in total amount to that contained in from 0.5 to 2.9 cc. of the serum of the rabbit.

This evidence, taken with that of Hektoen and Carlson (1910), Dreyer and Ray (1909) and many others, justifies the view that with the development of circulating antibodies a certain amount appears in the tissue spaces. As the antibody titre of the blood falls, there is a progressive loss of extravascular antibody, the fall probably lagging somewhat behind that of the blood. Apart from this accumulation of antibody in the tissue spaces and the rapid production of further antibody in response to a specific secondary stimulus, there is no evidence of the acquisition of antitoxic immunity by the tissues themselves.

THE IMMUNITY OF SNAKES TO THEIR OWN VENOMS

The very high immunity of poisonous snakes to their own venoms was discovered by Fontana (1781), who indeed thought it absolute, but the later observations of Bernard (1857), Mitchell (1861), Fayrer (1874), Waddell (1889) and Phisalix (1903) showed that the injection of sufficiently large doses of venom was followed by typical symptoms and death.

The Australian venomous snakes possess an extremely high immunity both to their own venoms and to those of closely allied species.

The venom of the Australian tiger snake (*Notechis scutatus*) is one of the most potent of snake venoms. The minimal lethal dose per kilo injected subcutaneously varies in mammalian species from about 5γ to 400γ of the dry venom, yet the injection of 1.4 gm. per kilogram

of this venom into a tiger snake causes only slight symptoms. The minimal lethal dose for the tiger snake of the allied, though somewhat less toxic, venoms of the copperhead (*Denisonia superba*) and death adder (*Acanthophis antarcticus*) is 1.0 gm. per kilogram, but that of the venom of the Indian Cobra (*Naia naia*) is only about 30 mg.

It is interesting to compare the resistance of the tiger snake per kilogram of body weight with that of the guinea-pig. Against its own venom the immunity of the tiger snake is approximately 180,000 times, against the venom of the copperhead 33,000 times, against that of the death adder 13,000 times, against that of the black snake 12,000 times and against the venom of the cobra 250 times that of the guinea-pig.

Other Australian venomous snakes behave similarly, but the immunity of non-venomous snakes is much less striking and qualitatively different. The carpet snake—*Python spilotes varigatus*, though it has a resistance against the venom of the Indian cobra of the same order as that of the Australian venomous snakes, has only about a fifth of their resistance to the common Australian snake venoms.

The same two factors which, in animals actively immunised against a single venom, appear to determine its resistance to other venoms, namely, antigenic relationship between the venoms and similarity of their toxic constitution, also play a part in the immunity of serpents. Nevertheless, the nature of their immunity to snake venom appears to be wholly different from that actively acquired in immunised animals.

Venomous snakes, as Phisalix and Bertrand (1893) showed, possess a not inconsiderable humoral immunity. The plasma of snakes is toxic, but this toxicity is removed by heating to 58°C. for half an hour, and the heated plasma is then found to be antitoxic. Now if venom be added to plasma and the mixture be heated, a certain amount of the venom will be neutralised by the plasma and a certain small amount of the added toxicity will be destroyed by the subsequent heating. If, therefore, venom gains entry into the circulating blood of a reptile, its presence should be displayed either by diminution of the neutralizing power of the heated plasma (if only small amounts of venom have gained access) or by the development of toxicity (if larger amounts have entered the circulation).

The antivenomous substances in the plasma of snakes cannot be identified with antitoxins produced in mammals following the injection of sublethal doses of venom. Grasset and Zoutendyk (1931), in a wide range of South African reptilia, found a very limited and trivial response in the production of antitoxins to tetanus and diphtheria toxins associated with a high but not absolute resistance to these poisons. In contrast, South African elapine snakes exhibited a fair response in the production of agglutinating antibodies against *B. typhosus* and meningococci.

Although venomous reptiles are capable of a limited immune response to antigens so dissimilar to the antigenic components of their own tissues, it would not be anticipated that any appreciable immune response would be obtained to the toxic proteins of their own venoms or of those from closely allied species. If the antivenomous substances in their plasma were indeed of the same kind as antitoxic antibodies produced in response to the injection of these venoms in mammals, the reptiles should be capable of further rapid production of antibody following the specific secondary stimulus of an injection of their own or of a closely allied venom. We have never succeeded in demonstrating such a response.

The protective power of the plasma from a tiger snake, into which a dose of 20 mg. of copperhead venom had been injected intravenously, was tested before the injection and on the fourth, sixth, ninth and fourteenth days afterwards. The smallest volume of plasma which mixed *in vitro* with 0.06 mg. of copperhead (four certainly lethal doses) rendered it non-lethal, when injected into guinea-pigs of 200 grammes weight, was before the injection 0.06 cc., on the fourth day 0.3 cc. and on the sixth, ninth and fourteenth days 0.1 cc.

The substantial nature of the immunity of venomous snakes is shown not only by the fact that they are able to withstand large doses of venom injected subcutaneously, which are absorbed only slowly and allow time for reserve protective mechanisms to come into operation, but they can also survive without symptoms the intravascular injection of doses of venom of the order of 50 milligrammes. The question at once arises as to whether antivenomous action of the plasma is alone sufficient to account for this immunity.

By bleeding out and subsequent thorough perfusion of snakes it was found that the total protective effect of the whole of the plasma

was equivalent to less than one-sixth of that dose of venom which could be injected intravascularly without the production of symptoms. Additional evidence that tolerance does not wholly depend upon the protective power of the plasma is provided by the regularly occurring increase in the toxicity of the plasma produced by the intravenous injection of small doses of venom.

This added toxicity of the plasma which is due to unneutralized venom disappears in the course of two or three days. The disappearance of toxicity occurs equally rapidly in snakes from which the venom glands have been removed or in which the Wolffian ducts with the afferent renal veins have been tied. The added venom is not selectively removed either by the venom glands or through the urinary tract.

In experiments of this kind within 4 hours, half, and within 26 hours, three-quarters of the added toxicity of the plasma had been lost. After 72 hours the toxicity of the heated plasma was usually of the same order as that of plasma obtained before the injection of venom.

The disappearance of venom from the circulating blood might be due to destruction by an enzyme in the plasma, or, as Phisalix claims to be the case when viper venom and viper serum are mixed and heated to 58°C., the plasma might render the venom heat-labile. Mixtures of sterile tiger-snake plasma and copperhead venom incubated *in vitro* for varying periods up to 20 hours at 17.5°C. and tested, both unheated and after heating to 58°C. for half an hour, showed no change in toxicity. It is evident, therefore, that Australian venom does not become heat-labile in contact with snake plasma, nor does the plasma contain an enzyme capable of destroying added venom.

Experiments in which venom was mixed with aseptically perfused and minced snake tissues suspended in sterile Ringer's solution at 0°C. for varying periods up to 40 hours showed losses in the toxicity of the supernatant fluid from 30 to 70 per cent. Controls in which washed snake red blood corpuscles were suspended in Ringer's solution and venom added showed no loss of toxicity. The loss of toxicity might be due to the fixation of venom by the tissues or to its destruction by tissue enzymes, but the value of the observations was to some extent discounted by the fact that similar results were obtained with the perfused tissues of a highly susceptible animal—the guinea-pig.

In other experiments after thorough perfusion of the intact reptile, a

limited amount of Ringer's solution containing gum arabic and in which the washed red blood corpuscles were suspended was kept in circulation by the heart, which continued to beat strongly. A reservoir received the outflow from the inferior vena cava and supplied the inflow into a cannula directed heartwards in the same vessel. Such preparations could be kept going with natural respiration and strong heart action for 2 or 3 hours, during which there was progressive oedema of the tissues and loss of fluid from circulation. They were extremely sensitive to venom, doses of the order of 5 mg. (which produced an effective concentration in the circulating fluid of about 0.07 mg. per cubic centimeter) causing cessation of respiration and necessitating oxygenation of the fluid in the reservoir. At the same time the reptile became paralysed and ceased to respond to stimuli. This effect passed off within one or two hours, when the snake again breathed naturally and responded to stimuli. In snakes with intact circulation a dose of 40 mg. may be injected intravenously without the occurrence of symptoms. This, allowing for the neutralisation of 4 or 5 mg. by the antivenomous substances in the plasma, would provide an immediately effective concentration of circulating venom of 1 or 2 mg. per cubic centimeter. The abnormal conditions of our perfusion experiments greatly diminished the natural resistance of the tissues to venom but the moderately rapid recovery from the symptoms of severe evenomation must have been due to the liberation or production of a further supply of antivenomous substances, to the neutralisation or destruction of venom by the tissues or to its elimination from the body.

Other conditions in which the tissues became more sensitive to venom were provided in experiments in which the aorta and vena cava were ligated at the cranial pole of the liver. The posterior half of the body was poorly supplied with blood through collaterals between the vessels of the body-wall and those of the spinal cord and lungs. After this procedure, which can be carried out without opening the air sac, the reptiles survived about two days in active condition and died on the third or fourth day. Injection of 10 mg. of venom into the posterior circulation caused paralysis spreading from the tail to the level of the ligatures, but a dose of 20 mg. of venom injected into the anterior circulation was tolerated without any ill effects.

These experiments suggested that the liver played no important part in the detoxication of venom, and in this connection it may be noted that much less venom was lost when put up *in vitro* with minced snake liver than with other ophidian tissues.

It may be concluded from the evidence so far presented that the immunity of serpents to their own venoms does not depend upon inability of the tissues to fix venom, because if venom is present in sufficient concentration in the circulating blood all the symptoms of envenomation observed in less resistant animals occur. It is only explained in part by the presence of circulating antivenomous substances. The rapid disappearance of venom from the circulation of the intact reptile indicates that the snake has some means of destroying or eliminating it. It is unlikely that this loss of toxicity of the plasma is in reality due to the mobilisation of further antivenomous substances, since there is no evidence of the persistence of these in excess. Elimination by the activity of the venom glands and Wolffian bodies has been excluded, but excision of the whole alimentary tract presented difficulties too great to be overcome. There remained the possibility that snake tissues contain an enzyme capable of destroying or detoxicating large quantities of venom.

Tiger snakes, the venom glands of which had been excised more than two years earlier, were bled out under ether anaesthesia and extracts of their skeletal muscle in 0.7 per cent saline were made aseptically by grinding the minced tissue with powdered quartz. After prolonged centrifugation the supernatant fluid was tested by mixing it with copperhead venom and testing the toxicity of the mixture immediately and after standing for 13 and 22 hours at 17.5°C. The controls were a solution of venom of the same concentration in saline, a mixture of venom in the same concentration with muscle extract heated to 100°C. to destroy the hypothetical enzyme, and a similar extract of guinea-pig muscle made up with venom to the same concentration. These and the experimental mixtures, which were shown to be free from gross bacterial contamination, were injected subcutaneously into mice, after suitable dilution, using groups of 20 or 30 animals for each dose. The doses were calculated per 20 grams of body weight for each mouse in terms of the amount of venom originally present. The extracts, both heated and unheated, were shown

to be devoid of toxic activity in twice the largest dose used in any of the tests.

The results of such an experiment (Table I) showed some loss of toxicity in the mixture of snake muscle extract and venom immediately injected. This was probably due in part to antivenomous substances in traces of plasma present in the extract and possibly to antivenomous substances other than a destructive enzyme from the muscle itself—though it should be remembered that after injection an enzyme, if present, would have opportunity for action in the bodies of the test animals. During the period of incubation at room temperature there were further losses, so that after 22 hours the results of the titration indicated that at least half of the venom present had been

TABLE I

Enzyme action of snake muscle extract. Per cent mortality in mice injected with mixtures of the following with venom and incubated at 17.5°C.

DOSE OF VENOM	SALINE INCUBATED (13 HOURS)	GUINEA-PIG MUSCLE EXTRACT (13 HOURS)	SNAKE-MUSCLE EXTRACT HEATED TO 100° C. FOR 10 MINUTES (13 HOURS)	SNAKE MUSCLE EXTRACT		
				Not incubated	13 hours	22 hours
mg.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.025	40					
0.030	55	30	40	40		
0.040	77	55	100	53	37	
0.050					63	27

destroyed. In this case the enzyme present in the extract from 18 gm. of muscle must, in 22 hours, have accounted for about 8 mg. of venom.

These observations suggest that the immunity of snakes to their own and allied venoms depends in large part upon the presence in their tissues of enzymes capable of destroying or utilizing the venom. The high resistance of the tissues of the snake to venom and the presence of antivenomous substances in the plasma afford time for this mechanism to be effective in the final removal of venom from the circulation.

It is of some interest to trace the factors which may have operated in the evolutionary development of these protective mechanisms.

If venom or its constituents were intermittently or constantly present in the circulating blood of venomous reptiles, a high natural

immunity would be essential to their continued existence and species without such immunity would become extinct.

Waddel (1889) thought that reptiles might absorb their own venom from abrasions of the mucosa of the alimentary tract. Snakes normally swallow a good deal of venom in and with their prey, but after the oral administration of large doses of venom, though some is recovered from the excreta undestroyed on the fifth day, at no time during the intervening period was there demonstrable absorption of venom into the circulating blood.

Phisalix and Bertrand (1894–5) put forward the view that the venom glands secrete into the blood-stream a toxic internal secretion identical with venom or at least with some of its constituents. They stressed the similarity of the effects produced in experimental animals by the serum of the viper and by its venom, and showed that the removal of the venom glands in vipers resulted after an interval of 67 days in a loss of the toxicity of the serum.

Calmette (1895) maintained that the toxic substances in the sera of venomous snakes, though similar in action, are not identical with venom, since they are heat-labile. He suggested that the blood of venomous snakes contains "diastatic" substances of cellular origin which represent certain of the constituent elements of venom. He found that by repeatedly injecting non-lethal doses of cobra serum it was possible to immunise rabbits and guinea-pigs against several lethal doses of cobra venom. Stephens (1899) and later Noc (1934) showed that Calmette's antivenene could neutralize the lytic element in cobra serum, and Flexner and Noguchi (1903) found that antisera prepared against the sera of certain North American snakes neutralised the lytic action of the homologous venoms.

Before either of these views can be accepted, it is essential to provide evidence that the heat-labile toxic substances of the plasma are identical with or akin to venom.

The local effects which follow the subcutaneous injection of snake sera are very striking—congestion, haemorrhage, oedema and even necrosis resulting. Similar changes follow the intraperitoneal injection of the sera of the Australian elapine snakes, but they resemble much more closely the effects produced by the injection of a viperine venom than those following the injection of the colubrid venoms.

So far as Australian snakes are concerned, the following criteria are necessary to establish the presence of venoms or venom-like substances in the plasma:

(1) The demonstration of a curari-like action of the plasma.
(2) The neutralisation of toxic elements in the plasma by monovalent antivenene.
(3) Active protection against venom by immunisation with plasma.
(4) Positive serological reactions with homologous venom and immune serum from animals immunised with plasma, and conversely.
(5) Positive serological reactions with snake plasma and sera from animals immunised against homologous venom.

The chief difficulty in the application of these criteria rests in the fact that during capture and in captivity snakes frequently bite themselves. Their sera shortly after capture frequently contain venom, and to ascertain if venom is a normal and constant constituent of the plasma, the snakes must be kept isolated for some days before bleeding out to obtain serum or plasma for experiment. When this is done, no convincing evidence can be obtained of the presence of venom in the circulating blood. Snake plasmas obtained with these precautions have no demonstrable curari-like activity, nor, as Arthus (1912) has also shown in the case of tiger and black snake sera, have they any coagulant action.

Monovalent tiger-snake antivenene affords no protection against a lethal dose of tiger-snake plasma injected intraperitoneally. It is true that by high immunisation of guinea-pigs with tiger-snake plasma some degree of active immunity against the homologous venom may be obtained, but traces of venom in the large amount of serum used for immunisation may explain these and Calmette's similar results. The sera of rabbits immunised with tiger snake and other Australian snake sera did not neutralise the homologous venoms and these sera tested by complement fixation with the venoms failed to indicate the presence of any common antigenic constituents in snake sera and venoms.

The results of our experiments upon the effect of excision of the venom glands on the toxicity of the plasma were wholly negative. Sera obtained from tiger snakes up to two years after the excision of

the venom glands did not differ in toxicity from those of snakes with intact glands provided that these were kept isolated for a few days before their sera were tested.

The one certain method by which venom can gain access to the circulating blood is by bites, either self-inflicted or by other reptiles. Most Australian venomous snakes exhibit their annoyance by biting themselves if they can find no more convenient object into which to discharge their venom. In the field, snakes after capture almost invariably bite themselves and their fellow captives, so that if "milked" soon after being caught they usually yield but little venom. Their plasma also is highly toxic when injected subcutaneously, but this toxicity diminishes greatly if they are isolated for a few days. Snakes also sometimes bite through the floor of the mouth by closing the jaws with the fangs still partially erect.

Sufficient opportunity thus exists for the entry of venom into the circulating blood to render a high immunity necessary to the continued existence of the many species of venomous ophidians.

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ESSENTIAL IMMUNIZING ANTIGEN OF THE TYPHOID BACILLUS[1]

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The multiplicity of antigenic components in bacterial cells is definitely indicated by recent advances in immuno-chemistry. In particular two fractions of the cell have been studied, designated as P, protein, and S, polysaccharide or hapten, in the pneumococcus by Heidelberger and Avery (1), and in the typhoid bacillus by Furth and Landsteiner (2). Doubtless in these two fractions, there will be found, respectively, as many P antigens as there are protein entities and perhaps as many haptens as there are different polysaccharides. The term *hapten* is here used as defined originally by Landsteiner, as a substance which specifically precipitates immune bodies but does not in itself stimulate antibody production.

Our study of bacterial cells of various organisms has been mainly an endeavor to isolate what may be termed the essential active immunizing antigen. By this term is meant that fraction, or chemical, of the cell which stimulates in experimental animals a response such that their defense against live virulent organisms is enhanced.

Much of the work in the chemical fractionation of bacterial cells has been done on the pneumococcus. Briefly, there is now agreement that active immunity may be produced in white mice by an active form of the polysaccharide of Heidelberger and Avery, and that at least protective antibody may be stimulated by its use in human beings (3). At the present time the possible active immunity in man is being measured by reduction of pneumonia morbidity. Boivin, Mesrobeanu and Mesrobeanu (4), and a little later Raistrick and Topley (5) have demonstrated the essential immunizing antigen of Bact. aer-

[1] This is one of a series of studies carried out in part under a grant from the Influenza Commission of the Metropolitan Life Insurance Company, and in part under a grant from the Pneumonia Fund of Johns Hopkins University.

trycke. More recently Pennell and Huddleson (6) have isolated from Brucella an antigenic b-anthroquinone carboxylic acid in crystal form.

Many investigators have studied typhoid organisms with respect to fractions which stimulated antibody production. Since Furth and Landsteiner (2) have published an excellent review of the subject, and since the primary interest here is in active immunity, a complete bibliography of previous work will be omitted. It is pertinent that, in a review of the literature, no report has been found of a successful experiment on the production of active immunity against the typhoid bacillus in experimental animals by any antigen except the intact cell. Yet, if production of antibody against the whole cell be considered a measure of active immunity, then the work of many previous investigators on the various fractions of the typhoid bacillus has suggested the possibility of isolating an essential antigen. To our knowledge, however, injection of any fraction has not been shown to have immunized the experimental animal against live virulent organisms.

Undoubtedly in isolating the essential antigen of the typhoid bacillus the chief difficulty has been the lack of a satisfactory animal test. The mucin method, suggested by Rake (7) and used so successfully by the Army Medical School Staff (8) in measuring the differences in antigenicity of the typhoid organism, has overcome this limitation. The method was based upon the original observations of Nungester (9) and Miller (10) with the meningococcus, that the addition of mucin to a live culture, before injection, either increases the virulence of the organism or decreases the defense of the animal so that a smaller number of organisms causes a fatal infection. With this technic available, it therefore seemed feasible to attempt the isolation of an essential antigen from the typhoid bacillus.

The present report therefore describes an investigation of the active immunity-producing properties and some of the chemical characteristics of the fractionated typhoid cell.

MATERIALS

The task of obtaining dry antigenic typhoid cells was simplified by the work of Douglas and Fleming (11) in which they found that this organism dried from acetone was, if anything, more antigenic than the moist cell. In other words, the antigen was not soluble in acetone. Furthermore, Furth and Landsteiner were unable to confirm the observations of earlier workers that a fraction soluble

in 95% alcohol or other higher solvents was antigenic. Consequently, in the present study this information was applied in the following manner:

Strain 58 (S58) of typhoid bacillus was selected, inasmuch as preliminary tests at the Army Medical School had indicated that this strain in its morphological, cultural and immunological properties was a typically virulent smooth form highly antigenic for both mice and human beings. Cultures were grown on a double strength veal infusion (fat-free) agar in Kolle flasks at 37°C. for 22 hours. This growth was washed from each flask with approximately 10 c.c. of sterile normal saline. To the combined suspensions was added an equal volume of acetone. After thorough mixing and standing at room temperature for two hours, this suspension was centrifugalized for one hour. The cells were again shaken with one-half the original volume of acetone and incubated at 37°C. for 12 hours. The cells were then collected and dried *in vacuo*. They will be referred to throughout the paper as "standard cells."

METHODS

Methods of chemical analysis used in this report are the conventional ones with the exception of estimation of acetyl content. This technic will be reported elsewhere. Immune horse serum was used for estimating precipitinogens. Tests for active immunity in mice were run as follows: a logarithmic series of dilutions of each preparation[2] was administered to mice in groups of five (or more, as given in the tables below). Swiss mice of an average weight of 16 to 18 grams were given two injections of 0.5 c.c. intraperitoneally 7 days apart. Seven days later the animals were tested for active immunity by injecting intraperitoneally live organisms (S58) suspended in sterile 6% mucin. The dose of live organisms is stated in each experiment below. The period of observation of the mice was 72 hours. The number of mice surviving is reported. In all experiments described, unvaccinated normal mice were simultaneously given 1000 live organisms intraperitoneally; without exception these died within 48 hours.

EXPERIMENT 1. COMPARISON OF LIQUID VACCINE AND "STANDARD" CELLS

In view of the work of Douglas and Fleming, a comparison was made between the acetone-extracted dried typhoid bacilli S58 and the heat-killed tricresolized vaccine prepared from the same strain. The resuspension of the acetone-dried organisms (standard cells) contained 3.6×10^{12} cells per cubic centimeter and the vaccine contained 10^9 cells per cubic centimeter. Dilutions of each of these were tested in mice in groups of five against 50,000 live organisms according to the technic described above.

[2] In order to remove any possible residual bacteria, before final precipitation, a solution of each sample was passed through a Seitz filter.

From table 1, it may be noted that the liquid vaccine protected 3 of the 5 mice against 50,000 live organisms (at least 50 M.L.D.) when 10,000,000 organisms were used as the vaccine dose; conversely, the acetone-extracted cells protected 3 of 5 mice against the same dose of live organisms when 360,000 "standard cells" were used for immunization. This is one experiment taken at random from several which were practically identical in results. Consequently it would appear that per cell the acetone-extracted typhoid bacilli were superior in antigenicity in mice to the liquid vaccine made from the same strain S58. Thus the results confirm the observations of Douglas and Fleming.

TABLE 1

*Comparison of liquid vaccine and "standard cells." Active immunity in mice**

	UNDILUTED	DILUTION				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Liquid vaccine:						
Survivals of 5 mice..	5	5	3	1	0	0
Number of organisms.	10^9	10^8	10^7	10^6	10^5	10^4
	10^{-4}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Standard cells:						
Survivals of 5 mice..	2†	3†	5	5	3	2
Number of organisms.	3.6×10^9	3.6×10^8	3.6×10^7	3.6×10^6	3.6×10^5	3.6×10^4

* Immunity was tested by inoculating 50,000 live organisms.

† The other mice in this group died before receiving the infective inoculum.

EXPERIMENT 2. POLYSACCHARIDE FRACTION PREPARED FROM BROTH BY THE CALCIUM PHOSPHATE METHOD

One method used to isolate the essential antigen from the typhoid bacilli was the calcium phosphate procedure for precipitating bacterial polysaccharides from broth culture (12). Tests on three preparations, 1A, 5A, and 6B, are included in this experiment. *1A.* Without ascertaining optimal conditions for precipitating the polysaccharide of the typhoid bacillus, the following technic was carried out for preparation of sample 1A:

One hundred liters of an infusion-free broth were seeded with a 6-hour culture of S58 typhoid bacillus and incubated at 37°C. for 22 hours. After adding 25 c.c. of a 4-normal NaOH solution to each liter, the mixture was agitated, allowed to

stand at room temperature for 2 hours, and then neutralized to pH 7.2 with 4-normal HCl. Then for each liter were added: 10 c.c. of 4.6% anhydrous Na_2HPO_4, and 20 c.c. of 10% $CaCl_2$ in a saturated $Ca(OH)_2$ solution. After allowing the heavy flocculent precipitate to settle for 2 hours, and discarding the supernatant fluid, the precipitate was collected on a hardened filter paper over night. The precipitate was dissolved in 4-normal HCl up to 4 liters. Then 4 liters of alcohol and 1 of ether were added to the mixture. After standing at 4°C. over night, a heavy precipitate settled out. The supernatant fluid was decanted, and to it two volumes of alcohol were added. A very fine homogeneous precipitate formed, which was insoluble at neutral pH but was soluble in acid. It was dissolved in 4-normal HCl and reprecipitated with 3 volumes of alcohol, and then combined with the first alcoholic precipitate above. The combined precipitates were dissolved at pH 7, the insoluble was centrifugalized out, and

TABLE 2

Polysaccharide fractions prepared from broth by calcium phosphate method

SAMPLE	TEST	PRECIPITIN TITER	NUMBER OF MICE	MOUSE IMMUNITY (SURVIVALS)						NITROGEN	GLUCOSE
				Dilution of samples							
				10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
										per cent	*per cent*
1A	1*	1:64,000	12	8	5	2	4	2	2	14.86	32.3
Standard cells	1*	1:16,000	6	2†	6	6	6	6	6	15.4	12.6
5A	2*	1:16,000	5	4†	2	0	2	0	0	10.75	27.9
6B	2*	1:8,000	5	0†	1	1	0	0	0	2.02	53.4
Standard cells	2*	1:16,000	5	2†	3†	5	5	2	2		

* The mice in Test 1 received 1000 living organisms; those in test 2 received 50,000.

† The other mice in this group died before receiving the infective inoculum.

the supernatant fluid reprecipitated with 3 volumes of alcohol. This precipitate was washed and dried *in vacuo*. Yield one gram.

This product was further purified by suspending in 200 c.c. N/10 acetic acid. The insoluble material after centrifugalization was suspended in chloroform and refluxed on the water-bath for 30 minutes to denature proteins present. Most of the protein was coagulated by this treatment and could then be easily separated from the active soluble material. To this soluble fraction after cooling 1½ volumes alcohol were added. The precipitate formed was combined with the acetic-acid-soluble fraction and neutralized to pH 7. After centrifugalization, the soluble fraction was precipitated with 3 volumes of alcohol. This neutral precipitation was then repeated; and after standing at 4°C. over night, the product was collected, washed and dried *in vacuo*.

In the preparation of sample 5A, the same general technic was employed as in

1A up to the first drying, except that the culture was grown in veal infusion broth for 8 days, and that $CaCl_2$ was not used in the initial precipitation.

In the preparation of sample 6B, a portion of the calcium phosphate precipitate from 5A was dialyzed for 48 hours against running water, and then precipitated with alcohol successively from acid and neutral solutions. The neutral solution was purified by repeated filtration on a Seitz filter and reprecipitation with alcohol.

Table 2 indicates the precipitin titer of these samples as well as of the standard cells, chemical estimations, and the survivals in mouse immunity tests. It would seem from the results shown in this table that nitrogen or reducing sugar content alone did not markedly influence antigenicity. It is also apparent that, while the calcium phosphate method as used here precipitated a substance polysaccharide in nature, it was similar to the typhoid hapten fraction described by Furth and Landsteiner (2), Spassky and Danenfeldt (13), Nichols and Smith (14), and others.

EXPERIMENT 3. COMPARISON OF INFLUENCE OF CONCENTRATED AND OF NORMAL HCl

In work on other organisms it has been found that the use of concentrated HCl results in an ideal separation of the constituents of the cell. With some organisms the essential antigen is greatly reduced in activity by this technic. For that reason, this experiment was carried out to ascertain the influence of concentrated and normal HCl on the stability of the active typhoid antigen. Since it has been found that reduction of salt content of the bacterial cell by dialysis facilitates the isolation of cellular fractions, in this experiment six grams of the acetone-extracted organisms were dialyzed 24 hours, made up to 300 cc. and divided into two aliquot parts.

(A) At room temperature an equal volume of normal HCl was added and held for 2 hours. The acid insoluble was separated and added to the acid insoluble of (B) below. To the clear acid supernatant fluid 3 volumes of alcohol and one of ether were added. The heavy precipitate which formed was collected. Another volume of alcohol and 2 volumes of ether were added to this supernatant fluid and the mixture placed in the icebox for 24 hours. The resultant precipitate was collected and washed and dried *in vacuo*—designated fraction 3C. The heavy precipitate with first addition of alcohol-ether was dissolved at pH 7, passed through a Seitz filter, and divided into 2 equal parts. One was reprecipi-

tated with 2 volumes of a fifty-fifty mixture of alcohol and ether. The precipitate was collected, washed and dried *in vacuo*—designated as 3A. The second part was refluxed with chloroform for one hour, placed in the icebox for 24 hours, filtered, and the filtrate precipitated with an equal volume of a fifty-fifty mixture of alcohol and ether. This precipitate was collected, washed and dried *in vacuo* —designated as 3B.

(B) In the cold (4°C.) an equal volume of concentrated HCl was added to the other portion of dialyzed material and held for 2 hours. The acid insoluble was collected, added to the acid insoluble from (A), treated with normal KOH, centrifugalized, and the clear supernatant fluid precipitated with 3 volumes of

TABLE 3A

Chemical and precipitin tests on immunizing fractions prepared by treatment with normal and concentrated HCl

SAMPLE	NITROGEN	GLUCOSE	PRECIPITIN TITER									
			1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000	1:256,000	1:512,000
	per cent	*per cent*										
Normal HCl:												
3A	5.08	9.6	+	+	+	+	±	T*	T	−		
3B	10.65	20.0	+	+	+	+	T	T	T	−		
3C	7.46	8.8	+	+	+	+	+	T	T	−		
Concentrated HCl:												
3D	9.08	12.4	+	+	+	+	+	T	T	T	−	
3E	6.13	14.4	+	+	+	+	+	T	T	−		
3F	1.56	0.0	+	−					·			
Insoluble residue treated with KOH	1.89	33.0	+	+	+	+	T	T	−			
Standard cells			+	+	+	+	+	+	±	±	±	±

* T indicates turbidity but no precipitate.

alcohol. This precipitate was collected, washed and dried *in vacuo*—designated as 3G. Identical procedures on the acid soluble material were then carried through with the concentrated HCl as with normal HCl in (A) above, except that all steps were carried out at 4°C. Fractions are designated as 3D, 3F, and 3E, respectively.

In table 3A are given the precipitin titers, and chemical estimations on these fractions, and in table 3B, the active immunity in mice. Two injections of antigen were given, and a dose of 1000 live organisms

to test for active immunity. In table 3B, the second test is the same except that the infecting inoculum was increased to 50,000 live typhoid bacilli.

A study of the results shows that the immunizing substance is stable in concentrated HCl in the cold and in normal HCl at room temperature. The endpoint of active immunity was not reached with either 1000 or 50,000 infecting organisms, inasmuch as the majority of the animals survived when the dilution given was 10^{-8} or 0.00001 mg. of the best fractions 3C and 3D. However, as seen in this table and also in table 5, it would appear that the material isolated with

TABLE 3B

Immunity tests on fractions prepared by treatment with normal and concentrated HCl

SAMPLE	TEST (1)—1,000 LIVING ORGANISMS SURVIVALS OF 4 MICE						TEST (2)—50,000 LIVING ORGANISMS SURVIVALS OF 5 MICE					
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Normal HCl:												
3A	0*	3*	4	4	4	2	2*	5	5	4	1	1
3B	3*	4	4	4	2	2	5	5	3	2	1	1
3C	2*	2*	3	4	4	4	1*	4*	5	5	4	5
Concentrated HCl:												
3D	0*	3*	4	4	4	4	0*	3*	4*	5	4	3
3E	2*	3*	4	4	2	2	3*	4*	5	4	1	0
3F	3*	4	3	2	3	1						
Insoluble residue treated with KOH	4	3	1	2	3	2						
Standard cells	1*	4	4	4	3	2	2*	3*	5	5	2	2

* The other mice in this group died before receiving the infective inoculum.

concentrated HCl is somewhat superior in immunizing activity to that prepared with normal HCl. It may be noted that the glucose number is low in both fractions 3C (8.8 per cent) and 3D (12.4 per cent), and the nitrogen relatively high, respectively, 7.46 and 9.08 per cent. It also may be noted that when these fractions are compared with the standard cells, 3C and 3D were immunogenic to as great or even greater extent per unit of weight. Fraction 3G is of interest, inasmuch as it represents the fraction soluble in KOH; and although the precipitin titer was one-half (1:8000) that of samples 3C and 3D, the active immunity was markedly less.

EXPERIMENT 4. COMPARISON OF 24- AND 72-HOUR TRYPSIN DIGEST

In this experiment the effect of trypsin digestion upon the typhoid bacilli as well as time of digestion were investigated by means of the following technic:

Two grams of the acetone-extracted cells, one gram of $NaHCO_3$ and one gram of trypsin were suspended in 100 c.c. water and incubated at 37°C. for 24 hours and 72 hours, respectively. Except for the time of digestion, the same steps of procedure were carried out for each. The suspensions after digestion were acidified to pH 2 and precipitated with 2 volumes of a fifty-fifty mixture of alcohol and ether. The precipitate was collected, washed and redissolved at pH 7. The insoluble material was removed and the clear supernatant fluid reprecipitated with 2 volumes of a fifty-fifty mixture of alcohol and ether. This precipitate was collected, washed and dried *in vacuo*. The fractions designated as 4A and 4B were obtained from the 24- and the 72-hour digestions respectively.

TABLE 4

Comparison of 24 and 72 hour trypsin digest

SAMPLE	NITROGEN	GLUCOSE	PRECIPITIN TITER	IMMUNITY—50,000 LIVING ORGANISMS SURVIVALS OF 5 MICE					
				10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	per cent	*per cent*							
24-hour 4A	5.0	32.4	1:64,000	0*	4*	4*	4	4	0
72-hour, 4B	4.08	31.3	1:128,000	0*	4*	5	3	1	0
Standard cells	15.4	12.6		2*	3*	5	5	2	2

* The other mice in this group died before receiving the infective inoculum.

Table 4 indicates the results in the precipitin and chemical tests as well as the survival of the mice in immunity tests. The usual procedure to test for active immunity in mice was employed, with an infecting dose of 50,000 live organisms. The two fractions showed no great difference in antigenicity. As might be expected, however, the 72-hour digestion removed more protein nitrogen—4.08 per cent as compared with 5.0 per cent in the 24-hour digest sample. In a comparison with the results of the previous experiment, it is seen that although antigenicity is approximately the same in both instances, the tryptic digestion does produce material of lower nitrogen and higher hydrolyzable sugar than results from the method employing HCl.

EXPERIMENT 5. TRYPSIN DIGESTION—SECOND EXPERIMENT

Because of the success of the previous experiment, trypsin digestion was used in another experiment for preparation of a larger quantity of material. Essentially the same procedure was carried out, except that purification was carried through more reprecipitations from filtered material. The final material was tested for nitrogen, phosphorus, sulfur, acetyl groups, hydrolyzable sugar, as well as for precipitin titer, and immunizing power in mice in both single and double injections, and in comparison with the best fractions 3C and 3D of experiment 3 above.

In detail the experiment was carried out as follows: six grams of standard cells were digested with 1 per cent trypsin in a 1 per cent solution of $NaHCO_3$ for 72 hours at 37°C. After centrifugalization, the supernatant fluid was acidified to pH 2 with 4-normal HCl and precipitated with 2 volumes of fifty-fifty alcohol-ether mixture. This precipitate was dissolved at pH 7, passed through a Seitz filter, and again precipitated with 2 volumes of alcohol-ether, washed and dried *in vacuo*. Yield 250 mg.

The undigested portion was dissolved in 50 cc. concentrated HCl at 4°C. and precipitated with alcohol-ether; then dissolved at pH 7, filtered and again precipitated as above. Yield 85 mg.

The two fractions were pooled and dissolved in 300 cc. water, filtered, and after standing in the cold for 72 hours, the insoluble was removed by centrifugalization. The clear supernatant fluid was then precipitated with 2 volumes of alcohol. Yield 100 mg. This product was designated 7C.

In table 5 the results are given of precipitin titer, chemical tests, and active immunity tests in mice receiving single and double injections of the indicated dilutions of samples 3C, 3D, and 7C. To make this comparison of the three fractions, all mice received simultaneously 100,000 live S58 bacilli seven days after the single injection and seven days after the second of the two injections.

In a study of this table, it is seen that when a single immunizing dose of 7C in a dilution of 10^{-6}, or a total of 0.0005 mg. was given, one-half of the mice survived against 100,000 live typhoid bacilli (at least 100 M.L.D.). But when the mice received two injections, all survived with as small a quantity as that contained in a 10^{-7} dilution,

or a total of 0.0001 mg. of fraction 7C. The chemical determinations upon this fraction indicate that nitrogen content (1.6 per cent) alone does not determine the activity of the material.

A comparison of immunizing power of the total fraction recovered with that of the intact standard cells is worthy of further comment. By calculation, the 6 grams of cells used in this experiment would have provided approximately 600,000 immunizing doses for mice, while the 100 mg. of 7C recovered from this 6-gram amount would provide

TABLE 5

Trypsin digest—second experiment

SAMPLE		ACTIVE IMMUNITY—100,000 LIVE ORGANISMS SURVIVALS OF 6 MICE								PRECIPITIN TITER	GLUCOSE	ACETYL	NITROGEN	PHOSPHORUS	SULFUR
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}						
											per cent	*per cent*	*per cent*	*per cent*	
3C	Single injection					1	1	0	0	1:16,000	8.8		7.46		
	Double injection					1	1	1	0						
3D	Single injection					4	0	2	0	1:16,000	12.4		9.08		
	Double injection					6	0	0	0						
7C*	Single injection			6	4	4	3	2	0	1:64,000	42.9	1.9	1.6	1.3	0
	Double injection			6	6	6	6	2	0						
Standard cells	Single injection			6	4	2	0	2	0	1:32,000	12.0		15.4		
	Double injection			6	6	5	1	2	0						
Liquid vaccine	Single injection	6	1	0	0	0									
	Double injection	6	5	5	2	0									

* Yield from 6 grams of dried organisms was 100 mgm.

1,000,000 immunizing doses. Thus the 100 mg. of the final material contained 40 per cent more immunizing doses for mice than did the original 6000 mg. of standard cells.

DISCUSSION

The object of this study was twofold: first, to compare the activity of the dried typhoid cell freed in great part of its lipoid and fat constituents with that of standard liquid typhoid vaccine; second, the

main issue, to determine whether or not an active essential immunizing fraction could be isolated from this organism of equal or greater potency than the intact cell. Comparisons of pneumococci dried from acetone with fractions derived from the same cells had shown that many more immunizing doses were found in the polysaccharide fraction, representing 6 to 10 per cent of the total weight of the organisms, than were obtained in the sample of the dried cells (15). Extension of this study to typhoid bacilli has shown repeatedly that in both respects a like relationship does exist. Thus in the first place, our findings confirm the earlier results of Douglas and Fleming that the fat-free dried cells are apparently superior to liquid vaccine. Several explanations of this finding suggest themselves: (a) that the method of preparation of a liquid vaccine may decrease some of the activity—that is, the high temperature used to kill the organisms or the tricresol may alter the molecule of the essential constituent; (b) that the lipoids present, which have been reported as antigenically inert, may actually retard or inhibit the development of the active component; and (c) that there may be some other product developed during the growth of the bacilli which in some manner paralyzes the immunizing mechanism. The present study also indicates that these acetone-extracted cells constitute a convenient form for storing fully antigenic bacilli. From our observations no detectable decrease in immunizing power was noted over a period of one year.

In the second place, it is to be emphasized that in this preliminary report tests were made only upon fractions of the cell which may be considered polysaccharide in nature; other fractions were not tested. It would appear that the essential immunizing antigen was present in all samples studied, but in varying amounts. The fractions low in nitrogen content and high in glucose number were the strongest in active immunizing power. Sample 7C which had a nitrogen content of 1.6 per cent, phosphorus 1.3 per cent, acetyl 1.9 per cent, and negative sulfur, and a glucose number of 42.9 per cent, is similar chemically to the hapten described by Furth and Landsteiner. These investigators found their hapten substance to be devoid of antigenicity in the sense of stimulating antibody production in rabbits. On the other hand, our tests were for the measurement only of active immunity. In consequence, no direct comparison can be made. How-

ever, from the fact that when alkali was used during the process of isolation, all fractions were weakly if at all antigenic (active immunity), it would appear that there may be both active and inactive forms of this fraction of the typhoid organism. In other words, strong alkali destroys the immunizing capacity, as has been shown with Type I pneumococcus SSS by Goebel and Avery (16). However, it is not to be inferred that this alteration necessarily follows the saponification of any acetyl groups present in the molecule.

From the protocols in the tables it will be noted that when high concentrations of the samples tested were injected into mice, with some samples all the mice died following the initial injection, with others, approximately 50 per cent died, and with others none died. No attempts have been made to test the characteristics of this toxic material—that is, whether or not smaller doses would immunize the mice against the toxic dose. The first injection invariably produced ruffling of the hair followed by crowding together of the mice suggesting a reduced body temperature, but it was noted that the mice surviving this initial injection withstood the second dose with no visible reaction. This was particularly evident in experiments in which only half the mice died on initial injection. It is possible that this toxic material plays a significant rôle in the pathogenesis of this organism. On the other hand, the best immunizing sample 7C produced much less toxic action, and all of the mice injected with 10^{-3} dilution, the largest dose, survived. For this reason, it is apparent that, whatever the cause of the toxicity, the essential immunizing antigen at least in the doses used is free from this activity.

In view of the above experiments and others to be reported later, it is clear that at least for mice there is an essential active immunizing antigenic fraction of the typhoid organism. This organism may therefore be added to the list of organisms which may be so fractionated, namely, pneumococcus, aertrycke as found by Boivin, Mesrobeanu and Mesrobeanu, and later by Raistrick and Topley, and Brucella from which Pennell and Huddleson reported the isolation of a crystalline b-anthraquinone carboxylic acid which exhibits an active antigenicity.

CONCLUSIONS

An essential immunizing fraction of the typhoid bacillus has been isolated which is chemically polysaccharide in nature, and immuno-

logically more active than the total weight of the acetone-extracted dried typhoid bacilli from which it is derived.

This report is considered as part of the study on typhoid immunization by the Army Medical School Staff. We wish to express our appreciation to Colonel J. F. Siler for his generous coöperation in furnishing the typhoid organisms, the mice, and the personal services of G. F. Luippold for carrying out the immunity tests.

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PRECOCIOUS PUBERTY FOLLOWING MEASLES ENCEPHALOMYELITIS AND EPIDEMIC ENCEPHALITIS

With a Discussion of the Relation of Intracranial Tumors and Inflammatory Processes to the Syndrome of Macrogenitosomia Praecox

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It is a generally accepted doctrine that the development of the secondary sexual characteristics is dependent upon the ductless glands, and in the same way it is always assumed that precocious puberty is due to tumors or to functional disorders of these structures. This doctrine is, of course, very strongly supported and is quite correct in its essentials. Certain facts, however, seem to indicate that the nervous system may also influence sexual development. For example, cases have been reported from time to time in which injuries of various types involving the brain have been followed by precocious puberty. It is the purpose of this paper to call attention to these facts and to report three cases in which inflammatory processes involving the nervous system were followed by premature sexual development.

Case 1 (Ped. 99210). *Measles encephalitis with three weeks of somnolence in a girl of 6½ years. Mental deficiency and personality changes evident on recovery, soon followed by recurrent convulsions. Development of secondary sexual characteristics 4 months after the onset of the illness.*

S. S., a white girl of 10 years, was brought to the Harriet Lane Home for examination on October 9, 1936, because of convulsions and behavior disturbances which had been present since an attack of measles at the age of 6½ years. Her birth and early history were essentially negative. She had been vaccinated against smallpox at 2 years and had been given diphtheria toxoid at 3. She had also had pertussis at 3 years and varicella at 6 years, having recovered from both of these uneventfully. She had started school at 6 years and, at the time that measles interrupted her progress, was leading her class of 55 children.

In March, 1933, six months after starting school, the patient developed measles. The attack was not especially severe and she seemed to be recovering when, on the seventh day, her temperature rose to 106°F., she had a series of convulsions and became critically ill. For the next three weeks she remained in a somnolent condition, then began to improve. As improvement took place, it became apparent that the child was backward. In her mother's words, she was "like a little baby being born again." From then on, she failed to progress mentally as before, developed definite behavior disturbances and began having convulsions at intervals of two or three months. In July, 1933, four months after the attack of measles and one month before she attained the age of 7 years, her breasts began to develop rapidly and pubic and axillary hair appeared. At 9¼ years she started menstruating and menstruation had continued, with only slight irregularity, up to the time of examination.

Physical Examination showed a well nourished girl of 10 years, slightly above average height, who was restless and distractible, and laughed and talked in a high-pitched voice. Secondary sexual characteristics were well developed, as shown in the illustration. Otherwise there were no physical abnormalities, and a detailed neurological examination was also negative. Psychiatric examination revealed an intelligence quotient of 61.

Although the onset of menstruation is seen occasionally as early as 10 years of age in otherwise normal individuals, the rapid appearance of secondary sexual characteristics in this girl before the age of 7 years, within a very few months after measles encephalitis, leaves no doubt that the premature onset of puberty in her case was in some way related to the encephalitis, which had clearly caused permanent damage to the brain. Little can be said about the possible localization of the lesion in the brain. The prolonged somnolence, however, suggests that the hypothalamus was involved.

Case 2 (Ped. 87087). *Measles encephalitis associated with prolonged somnolence, blindness, and extensive paralysis in a girl of 9 years. Rapid recovery from the paralysis. Retrobulbar neuritis persisted. Personality changes were soon evident. Sexual development began 3 months after the onset of the illness.*

C. J., a white girl of 9 years, developed measles December 31, 1933. She was not very ill at first and on January 2, 1934, the rash was beginning to fade and her temperature had fallen to normal. The next day, however, she became very ill. The temperature rose to 105°F. and she became delirious. Violent generalized choreiform movements appeared with the onset of delirium. The following day she was in a deep stupor. The jaws were opened with difficulty because of trismus. There was retention of urine requiring catheterization. The stupor persisted for a week or more and was followed by somnolence which lasted in all

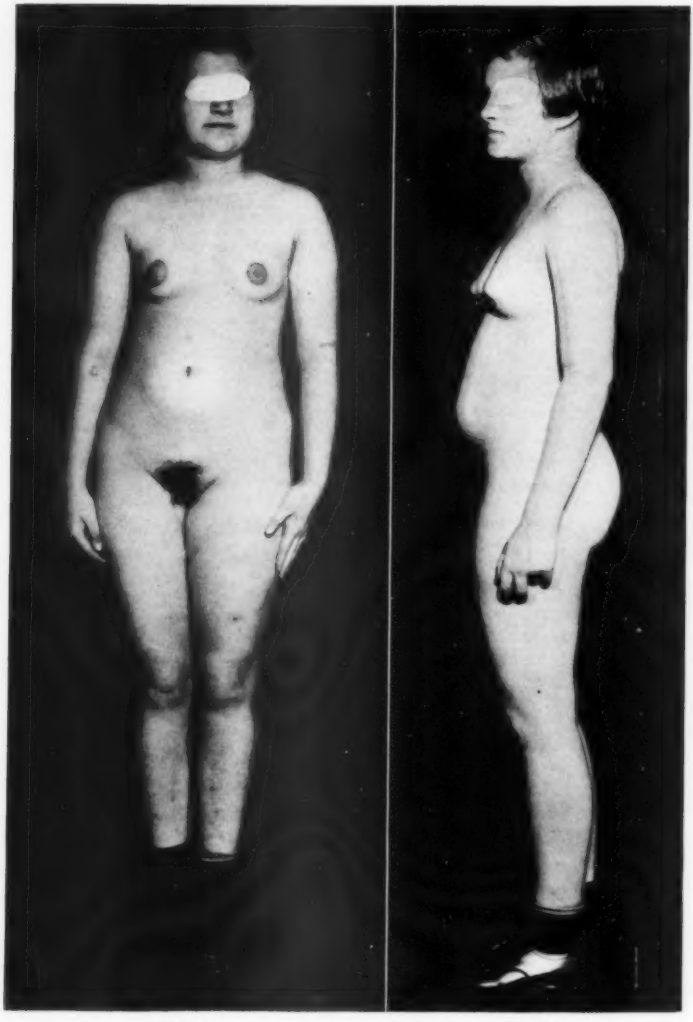

FIG. 1 FIG. 2

FIG. 1. PHOTOGRAPH OF PATIENT IN CASE 1. FRONT VIEW, SHOWING PREMATURE DEVELOPMENT OF THE BREASTS AND NIPPLES, THICK GROWTH OF PUBIC HAIR AND BODILY CONTOUR SIMILAR TO THAT OF AN ADULT WOMAN
FIG. 2. LATERAL VIEW OF SAME PATIENT

approximately a month. The patient was fed by gavage during this period. As the stupor cleared up, it was apparent that the extremities as well as the bulbar muscles were partially paralysed. The child seemed to be quite blind. During the fifth week, some return of power was apparent in the left leg and later the left arm began to improve. Recovery of the right arm and leg and of the bulbar muscles followed in rapid succession. During March the patient began to take a few steps and could use her arms well enough to feed herself. Speech was much altered. At this time vision was present but was still so much reduced that the patient could not recognize her parents. She was very restless and irritable. The least provocation led to screaming spells and temper tantrums.

By April the child's general condition was much improved. Her speech was clear and she could walk almost normally. Her breasts now began to grow rapidly, despite the fact that she was just 10 years old. Menstruation occurred a few weeks later. There was frequent masturbation.

The child was examined at the Harriet Lane Home on May 5, 1934. She was a tall slender girl of 10 years, rather restless and irritable but evidently of normal or more than average intelligence. The changes of puberty were already well advanced; there was some axillary and pubic hair; and the breasts were of adolescent type, with sharply pointed nipples. The right pupil was slightly larger than the left and reacted to light somewhat less promptly. Vision in the right eye was 20/80 and in the left eye 20/20. A large central scotoma was demonstrated on the right. The right optic disc showed outspoken temporal pallor and the left disc was slightly pale on the temporal side. Neurological examination revealed no other abnormalities, despite the history of extensive palsies during convalescence.

In February 1936 the child returned for reexamination of the eyes. Vision in the right eye had improved and was then 20/50. The scotoma had diminished in size. Her behavior was better, in that she was calmer and more coöperative. The breasts had grown larger and more mature since the last examination but were still those of adolescence, being pointed rather than round. Regular menstruation had continued.

The case is open to criticism in that the beginning of sexual development was not so accelerated as to be beyond the limits of normal. The rapid appearance of puberty so soon after a severe illness was so striking, however, that we feel justified in including this case. Prolonged somnolence was a striking feature of the acute stage of the disease, as it was in the first case, and suggests the possibility of involvement of the hypothalamus. The misbehavior was perhaps not due entirely to the measles encephalitis, for the patient's mother was a very irritable and unstable woman and may have transmitted some of her characteristics to the child.

Case 3. A boy of 11 years had epidemic encephalitis with fever, diplopia, and somnolence. During the next year, he developed transient obesity and polyuria and later a Parkinsonian syndrome with seborrhea and salivation. At the same time his sexual development rapidly advanced and was accompanied by hirsutism and misbehavior.

C. D., a white boy of 12 years was brought to the hospital in September 1921 because of sexual assaults upon little girls. The history was elicited that he had been a well behaved child until the fall of 1920 when, at the age of 11 years, he had had an illness characterized by fever, diplopia, and somnolence which had lasted about 2 weeks. He seemed to make a complete recovery and was able to return to school. For a few months after this he gained weight very rapidly and his parents noticed that he drank water in large quantities. Sleep was normal at that time. Soon it was noted that his facial expression was altered and that his movements were slower than they should be. When he returned to school after the Christmas holidays, he could make no progress, although he had been a fairly good student before his illness. Now he could not keep his attention on his studies. In August he was arrested in company with several other boys because of a sexual assault upon a little girl. It was claimed that the patient had persuaded the other boys to join him in the assault, and investigation revealed that the patient had been molesting little girls for several months.

Examination revealed a striking picture of precocious sexual development, almost identical with that found in the case of Horrax and Bailey, published in 1928. The boy was somewhat over-weight and there were numerous striae over his shoulders and abdomen which indicated that his weight had been much greater some time before. There was a heavy growth of body hair over the chest, abdomen and thighs, and the axillary and pubic hair was abundant and coarse. A soft growth of hair was seen on the upper lip. The penis and testicles were fully developed and seemed larger than normal in contrast with the patient's small body. Unfortunately, the prostate was not examined. The voice was changing, being at times deep and again of childish pitch. The patient showed the stooping posture and slow movements of the typical Parkinsonian syndrome. There was a well marked mask-face, unblinking stare, muscular rigidity with cogwheel phenomenon and lack of arm-swing in gait. There was, however, no tremor. The mouth was always full of saliva which drooled from the lips at times, and the face was always shiny and greasy because of seborrhea. The pupils reacted well to light and fairly well during accommodation.

During his stay of several weeks in the hospital the child was docile and good natured. He was always smiling and expressed excessive affection for everyone. Every morning he greeted the physicians with obvious pleasure as if they were old friends who had been away for a long time. He would get into bed and masturbate at frequent intervals during the day unless the nurse watched him closely. On several occasions during the night he crept into the female ward, which adjoined his ward, and attempted to crawl into bed with a young woman. The next morning he would express great regret, and would readily promise to

behave in the future. There was no alteration of sleep rhythm. No polyuria or polydipsia. After six weeks his condition and behavior were unchanged and he was discharged. His subsequent fate is unknown. It is to be regretted that no photographs were made.

DISCUSSION

In the complex and voluminous mass of information and misinformation devoted to the functions of the ductless glands, certain facts concerning precocious puberty would seem to be relatively clear. Premature sexual development in female children, involving both the external and the internal genitalia and associated with menstruation, may be caused by *tumors* composed of the *granulosa cells of the ovary*. In the same way, *testicular tumors* formed of *interstitial cells* may give rise to complete sexual development in male children. It seems to be assumed that such tumors produce sex hormones which under normal conditions are not secreted in significant amounts until the age of puberty.

Abundant observations have established the fact that tumors arising in the *cortex of the adrenal gland* are frequently associated with precocious sexual development in the male and with virilism in the female. The true origin of these tumors is still uncertain, but the cells of which they are composed resemble the normal cells of the adrenal cortex. Diffuse hyperplasias of the adrenal glands without true tumor formation have also been discovered in cases of sexual precocity.

Certain evidence, most of which has been derived from experimental work, has led to the belief that the *anterior lobe of the pituitary gland* elaborates secretions which play an important rôle in sexual development. Cushing has described a clinical picture including among its symptoms obesity, hirsutism, and hypertension, and resembling in some particulars the virilism associated with adrenal tumors. This picture he attributes to basophil adenoma of the hypophysis. The true significance of this condition is still uncertain. It must be said, however, that whatever the future may bring forth, not one single case has yet been described in which precocious sexual development has been definitely connected with hypophyseal tumors of any type.

The relation of *tumors of the pineal body* to precocious puberty is still

a subject of dispute. For many years the syndrome of macrogenitosomia praecox was regarded as diagnostic in pineal tumors. The early case reports were based upon very inadequate histological studies and any tumor in the pineal region was described as a pineal tumor. In recent years, studies by Dandy, Horrax, and Bailey have revealed that true pinealomas are rarely, if ever, associated with precocious puberty. In his comprehensive article, published in 1927, Haldeman included 113 cases which had been reported as instances of pineal tumors. Only 10 of these had been identified definitely as pinealomas, although adequate study would probably reveal that a number of pinealomas had been erroneously described as sarcomas or gliomas. In only 16 cases, all in males, was precocious puberty noted. Nine of the tumors in this group were classified as teratomas, two as hyperplasias, and one each as sarcoma, angiosarcoma, adenoma, neuroepithelioma and unclassified. Not one verified pinealoma was associated with the "pineal syndrome" of macrogenitosomia praecox. A case reported by Bailey and Horrax in 1928 (their case 3) is of great interest in this connection. The child began to develop sexually at the age of 3 years, and within a short time, the penis and testicles attained adult proportions, the voice assumed a deep masculine tone, pubic and axillary hair became abundant, the general muscular development became that of a man, and well marked obesity appeared. At the age of 11 years the child began to show signs of increased intracranial pressure. Death occurred after operation and at postmortem examination an astrocytoma was found in the third ventricle. The pineal body, the pituitary and the adrenal glands were histologically normal. The testes were fully developed. The significance of these facts is perhaps not entirely clear, but certain deductions seem possible. First, it is evident that there is little to support the theory that precocious puberty is due to excessive secretion of a sex hormone by the tumor. Since there is no uniformity in the histological characteristics of these tumors and since none of them show a glandular structure, it is almost inconceivable that they should all secrete hormones. It would also seem reasonable to assume that the symptoms cannot be due to destruction of the pineal body and loss of its hypothetical secretion, for in many instances, as for example in the *perfect* case of macrogenitosomia praecox of Horrax and Bailey, the

pineal body was not involved by the growth. The "pineal" tumors found in the cases of macrogenitosomia praecox have only one feature in common, *i.e.*, they arise in the general region of the pineal body or extend into this region. It is unfortunate that histological studies in such cases are confined to the tumor and that the brain is rarely studied. One feels confident in asserting that proper studies would reveal destructive changes in the walls of the third ventricle and in the hypothalamus, for pineal tumors almost always invade the third ventricle. From the facts available at present, one might conclude that the existence of the so-called pineal syndrome of macrogenitosomia praecox constitutes strong evidence *against* the diagnosis of pinealoma.

Some very illuminating facts have come to light in the study of certain tumors which have arisen in the hypothalamus and which have not been included in the literature dealing with pineal tumors. For example, Huyer, Lhermitte, and Vogt have published a careful study of the case of a male child who exhibited complete development of the sexual organs at the age of 9 years. At postmortem examination an "ependymoglioma" was found in the hypothalamus which had destroyed the mammillary bodies and the tuber cinereum. The pineal body, the adrenal glands, and the hypophysis were normal. Vickers and Tidswell have published an almost identical case. This was that of a boy who began to develop sexually at the age of 3½ years, and was soon sexually mature. An astrocytoma was found at autopsy, which had destroyed the mammillary bodies and the tuber cinereum. The pineal body, the hypophysis and the adrenal glands were normal, whereas the testes were enlarged and mature. Le Marquand's case is similar in every way. The boy showed the syndrome of macrogenitosomia and at postmortem examination a tumor in the floor of the third ventricle was found. The pineal body was not involved and the ductless glands were normal.

A few instances are on record in which inflammatory processes in the brain have been followed by precocious puberty. André-Thomas and Schaeffer describe the case of a boy of 12 years who had an illness thought to be meningitis at the age of 3 months. Hydrocephalus followed and the child was thereafter mentally defective. There was delay in walking and in talking. The development of the sexual

organs, however, was precocious and, when the boy was examined at the age of 12 years, the external genitalia were fully formed and there was abundant body hair of masculine distribution. Ventricular puncture was followed by purulent meningitis which proved fatal. At the postmortem examination well marked internal hydrocephalus was found. The third ventricle was greatly distended and there were destructive lesions of long standing in the tuber cinereum and adjacent portions of the hypothalamus. The pineal body was normal, as were the adrenal and pituitary glands. The testicles approached adult size and structure. Poos has reported a case of chronic tuberculous meningoencephalitis in which the sex organs began to develop at the age of 7 years and the penis and testicles had attained adult size a year later. There was also bitemporal hemianopia and bilateral papilledema. At autopsy tuberculous lesions were found in the walls of the third and lateral ventricles, and there was tuberculosis of the lymph nodes, the liver, and the spleen. The pineal body was normal. Schlesinger has reported a case which is almost unique, in that it occurred in a girl. There was a history of meningitis at the age of 4 months which was followed by hydrocephalus. At the age of 7 years the breasts began to grow, and sexual development was continuing when the child was examined at the age of 8 years and 8 months. She had, however, not begun to menstruate at that time. Neurological examination revealed mild optic atrophy, spasticity of the legs, and mental deficiency. The roentgenograms showed a normal sella turcica. The lack of postmortem examination in this case deprives it of much of its weight. The same objection applies to the case of Kwint which was that of a boy who had infantile cerebral paralysis at the age of 2 months and who developed sexually at 8 years.

It is well known that obesity, genital dystrophy and diabetes insipidus are not especially rare symptoms among the sequels of epidemic encephalitis. Sometimes these symptoms are transient and are eventually replaced by cachexia. In a careful anatomical study of a number of victims of this disease, Eaves and Croll found destructive lesions in the hypothalamus in all such cases. They concluded that the hypothalamus was more constantly and more severely affected in epidemic encephalitis than any other part of the brain,

with the single exception of the substantia nigra. Diabetes insipidus and obesity were found, as a rule, in association with less destructive lesions in the hypothalamus and cachexia was apt to be associated with more destructive processes. These writers found little to support the theory that such symptoms might be due to lesions in the hypophysis. The hypophysis was sometimes slightly damaged but the degree of injury bore no constant relation to the symptoms. In rare cases precocious sexual development, rather than genital dystrophy, has followed epidemic encephalitis. Stern has published the case of a boy who developed macrogenitosomia praecox as well as a severe Parkinsonian syndrome following encephalitis, and other less striking cases of the same type are on record. The fourth case of Walsh may be mentioned in this connection. Our own third case, given above, is even more remarkable than the case of Stern. Unfortunately, no postmortem studies have been made.

Scarcely any mention can be found in medical literature of precocious puberty following the disseminated encephalomyelitis of measles, vaccinia, and varicella. Boenheim, however, has mentioned one case in which a little girl of 7 years showed precocious development of the sexual organs, obesity, and periodic menstruation following an attack of measles encephalitis. The rarity of diabetes insipidus among the sequels of measles encephalitis would seem to indicate that the hypothalamus is not frequently involved in this process.

We can say very little by way of explanation for these observations. It seems to be clear that tumors and inflammatory processes involving the brain in the general region of the third ventricle, and especially the hypothalamus, may in rare cases be associated with precocious sexual development. We feel justified in saying that this syndrome has probably no relation to the pineal body whatever and the simplest interpretation of the facts is that the injury to the nervous system is in some unknown way responsible. It is, of course, quite possible, or even probable, that the injury to the brain has resulted in an altered innervation of some of the ductless glands. No doubt, a number of interpretations might be offered. It is of interest that almost all cases of precocious puberty found in association with cerebral lesions have occurred in male children. The significance of this fact is not apparent. Our first two cases represent possible exceptions to the

rule. It is impossible at present to venture any opinion about the exact location of the lesion or lesions responsible for this syndrome. The case of Huyer, Lhermitte and Vogt is probably the most important one in this connection, for the tumor was well localized and a very careful anatomical study was made of the brain. The posterior part of the tuber cinereum and the mammillary bodies were found to have been destroyed. Other cases, such as that of André-Thomas and Schaeffer, point to the same general region, although the process was not so sharply localized.

SUMMARY AND CONCLUSIONS

(1) Two cases of precocious sexual development in young girls following measles encephalitis are described and another case of premature development of the sex organs and of sexual misbehavior in a boy, following epidemic encephalitis, is given.

(2) A survey of the literature seems to indicate, we believe, that destructive lesions in the region of the pineal body and in the walls of the third ventricle may result directly or indirectly in the syndrome of macrogenitosomia praecox. Cases are cited in which this syndrome was associated not only with tumors arising in the region of the pineal body but with tumors of the hypothalamus. The same syndrome is known to follow inflammatory processes, such as meningitis, meningoencephalitis, and epidemic encephalitis.

(3) The information available at present indicates that the pineal body plays no significant rôle in the production of macrogenitosomia praecox, for this gland is quite normal in many cases in which this syndrome is fully developed and destruction of the pineal body may be followed by no apparent change in the genital organs. We have failed to find a single instance in which definite macrogenitosomia praecox has been associated with a verified pinealoma.

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REFLEX VESICAL CONTRACTION IN THE CAT AFTER TRANSECTION OF THE SPINAL CORD IN THE LOWER LUMBAR REGION[1]

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INTRODUCTION

A recent report discussed micturition in the cat after section of the sacral nerves (Langworthy and Hesser, 1936). Periodic waves of contraction of the muscle appeared during vesical filling and showed no tendency to summation. They could not be influenced by cutaneous or deep stimulation of the perineal region. The present study deals with the function of bladder contraction after transection of the spinal cord.

Reflex micturition after spinal transection has been of interest to many observers since the study of spinal man by Head and Riddoch (1917). Holmes (1933) and Denny-Brown and Robertson (1933) have pointed out that under these conditions it is subject to the play of many factors. In certain instances it is probably dependent upon local stimulation of the bladder muscle induced by the contraction of the abdominal muscles that follows reflex flexion of the legs.

In experimental work with animals it is possible to put the lesion at any desired level in the spinal cord. Preparations with transection in the lower lumbar segments are of particular interest. The cats are still able to use the hind-legs, although imperfectly. The abdominal muscles do not take part in any reflex activity. The sympathetic innervation to the bladder is given off above the level of the operation. The parasympathetic supply, which is of especial

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importance in controlling vesical contraction, is mediated through the isolated sacral portion of the cord.

METHODS

Eighteen female cats were used in the experiments; they were deeply anesthetized with nembutal at the time of each experimental procedure. A fine glass catheter was inserted into the bladder through the urethra; this was held in place by the tone of the urethral muscle, but fluid escaped around it when the bladder emptied. By means of a T tube, the catheter was connected with a source of fluid and with a water manometer. Graphic records were made by means of a tambour and kymograph. The kymograph ran at a fairly constant rate in all the experiments. The intravesical pressure in centimeters of water was noted upon the tracings. Oxycyanide of mercury in a 1 to 2000 dilution was used for filling the bladder. Control vesical readings were always made before the cord was sectioned.

The operation itself involved a dorsal midline incision and laminectomy of the fifth and sixth lumbar vertebrae. The dura was opened carefully and the dorsal roots were gently laid aside. The underlying cord was transected completely, care being taken to avoid injuring the spinal nerves. The cut was made in the region of the entrance of the seventh lumbar roots into the cord.

EXPERIMENTAL FINDINGS

If graphic records of bladder filling are made after section of the spinal cord in the lower lumbar region, the resultant changes are very similar to those described after section of the sacral nerves. During the first few days following the operation there is an increase in the vesical capacity. Later the capacity becomes normal or less than normal. At this time distinct waves dependent on muscle contraction appear in the record. These waves differ little from those observed after section of the sacral roots. The muscle responds very actively to stretch stimuli, and the contraction waves become more marked toward the end of filling. The intravesical pressure at the time fluid escapes spontaneously from the urethra is considerably decreased. These findings have already been described in a previous paper (Langworthy and Hesser, 1936).

The results at variance with those observed after section of the sacral roots have to do with responses of the bladder muscle to stimulation of the periphery of the body. In a normal animal a slight rise in vesical pressure can sometimes be obtained by similar stimulation. The phenomenon will first be described as seen in the unoperated cat. A rhythmical stimulus applied to cutaneous or deep structures, such as pinching with a clamp, is effective. The area from which responses can be elicited embraces the tail and perineum including the vagina and rectum. Occasionally, a slight rise can be obtained by rhythmically pinching the outer toe; stimulation of other portions of the body seldom elicits increase of vesical pressure. The smooth muscle contraction is graded quantitatively in relation to the portion of the responsive region which is stimulated. Thus a slight response is obtained from the tip of the tail, the contraction becoming progressively larger as the stimulus approaches the base of the tail. The largest contractions are obtained from the perineum, vagina and anus. Continuous stretching of the sphincter of the vagina or anus with a clamp will supply a particularly effective stimulus.

These responses to stimulation of the posterior portion of the body become greatly enhanced after transection of the spinal cord in the lower lumbar region. When the bladder has begun to show reflex activity, stimulation of the perineum or tail gives rise to marked vesical contractions. If the bladder muscle has previously been quiescent and a contraction wave is produced by stimulation, it is followed by rhythmical waves which continue for a considerable length of time. These responses are more expansive than those belonging purely to vesical filling. It is easy to see from the records that they are compound contractions which show an almost complete summation. The quiescent period between the rhythmical groups of waves is much longer than that which occurs between the waves seen during vesical filling after cutting of the sacral nerves. When the large waves have become established, further stimulation increases their height. Stimulation is relatively ineffective at the beginning of the quiescent period, but more effective as this period draws to a close.

The findings which have been outlined above are illustrated in the

figures. Responses on stimulation of the perineum in the normal cat are very easy to obtain when the anesthesia is relatively light. They are shown in figure 1, A. Thus a small rise of pressure was elicited from pinching the tip of the tail; larger responses were produced by spreading the vagina or the anal sphincter with a clamp. The contractions were of short duration.

The reflex waves were larger and more easily obtained 3 days after section of the spinal cord (fig. 1, B). The first two rises were obtained by stimulation of the tip of the tail, the last three by stimulation of the base of the tail. The responses from the base are seen

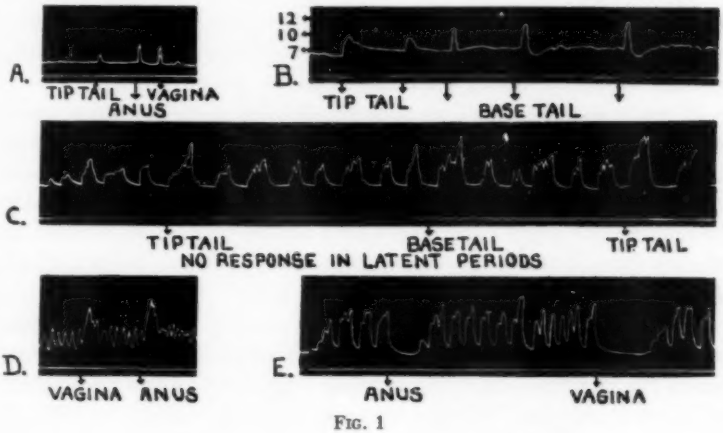

FIG. 1

to be of greater amplitude than those from the tip. Following the phasic contraction of the muscle, it is evident that the pressure fell below the previous base line of resting pressure. This can be seen particularly well in the last two responses. No fluid escaped from the urethra. Thus the increase of pressure was followed by a decrease below the previous level, after which the intravesical reading slowly rose to normal again. This record also shows that stimulation of the caudal portion of the body tends to produce rhythmical contraction waves which persist long after the stimulus has ceased. These waves appear toward the end of the record.

In figure 1, C, the rhythmical waves are larger. They tend to

increase in amplitude through the record as a result of the peripheral stimulation. It is quite evident that the waves are not simple contractions but are built up of an imperfect summation of a number of contractions. In certain portions of the record the summation is better than in others. The groups of contractions of the vesical muscle are separated by quiescent periods, which are longer than those developed between the simple waves that are associated with filling of the bladder. Stimulation of the perineum and tail increased the amplitude of the waves. During the latent period, peripheral stimulation was relatively ineffective.

Figure 1, D shows the effect of peripheral stimulation at a time when small waves of contraction were present. Stretching of the vagina or anus produced a summation of the waves and a strong, though poorly sustained, contraction of the vesical muscle.

It was also found that stimulation of the vagina and anus under certain circumstances would cause the waves of vesical contraction to cease, and the pressure to fall to a lower level. This response could never be obtained from the tail. This finding is represented in graph E of figure 1. The pressure fell at once on application of the stimulus, and the waves stopped so long as it was being applied. After it ceased, they were built up again slowly.

The type of response elicited on stimulation of the vagina or anus was determined by the condition of the bladder muscle at the time when the stimulus was being applied. This is apparent in graph A of figure 2 where increase and decrease of vesical activity on stimulation of the vagina may be observed in the same graph. The large rises of pressure in the graph are due to the introduction of fluid into the system. At the beginning of the experiment the vesical pressure was low. At this point stretching the vagina caused contraction waves of the bladder muscle. Later in the process of filling, the intravesical pressure remained high. At this point vaginal stimulation caused the pressure to fall rapidly. Cessation of stimulation was followed by the onset of rhythmical waves of contraction.

It was found that painful stimulation of the portion of the body supplied by the spinal cord above the level of the transection would cause the intravesical pressure to fall. The fall of pressure could be obtained from the abdomen, thorax and head. The adequate stimu-

lus was a painful one. The response was in general more difficult to elicit than the fall of pressure from stimulation of the rectum and vagina. Thus it was impossible in many cases to stop the rhythmical contraction waves.

Graphs B and C of figure 2 show the effect of stimulation of the skin of the abdomen. In graph B the intravesical pressure remained

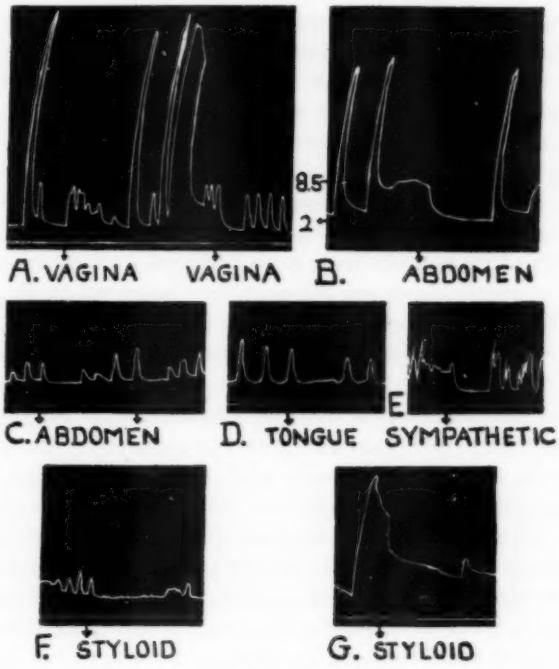

FIG. 2

high toward the completion of filling, and following the addition of an increment of fluid. Pinching the skin of the abdomen caused the pressure to fall and to remain at a low level. In graph C rhythmical waves of vesical contraction were present. Stimulation of the abdominal wall caused these waves to stop. After the stimulus was removed, the contractions returned and increased in amplitude.

In graph D of figure 2 is shown the effect of stimulation of the

tongue. The rhythmical waves of vesical contraction stopped completely during the period of stimulation. Pressure upon the styloid process produced a similar effect, as shown in graphs F and G of figure 2. In graph F the waves of contraction after a brief latent period stopped during the period of stimulation. The pressure remained high in graph G following the addition of extra fluid. Pressure upon the styloid process caused an abrupt fall of pressure. After the stimulus was removed the pressure rose again.

It is evident that painful stimuli applied to any portion of the body innervated above the section of the spinal cord may under certain circumstances produce a fall of intravesical pressure. The result is best obtained when intravesical pressure is high at the time of stimulation, and when the rhythmic contractions are not too strong. Increase of vesical pressure was never elicited from the areas innervated above the level of the cord section. The fall of pressure was obtained more easily from some preparations than from others. The sympathetic fibers are the only ones given off to the bladder above the level of the transection. The fall of pressure appears, therefore, to be dependent upon impulses carried along the sympathetics.

To test this assumption the sympathetic nerves to the bladder were exposed in these same cats with the spinal cord cut. Stimulation of the sympathetic trunks with an electric current using a shield electrode produced a fall of intravesical pressure similar to that obtained by applying nocuous stimuli to the surface of the body (graph E, fig. 2). The abdominal sympathetic trunks were then removed. Cutaneous and deep stimulation of the portions of the body innervated above the section of the spinal cord had no further effect upon the bladder activity.

DISCUSSION

Reflex micturition in the cat after transection of the spinal cord in the lower lumbar region differs from that observed after section of the sacral nerve roots in that the tone and contraction of the bladder musculature are markedly influenced by stimuli applied to the skin and deep tissues of the body. Thus micturition following transection of the cord possesses a further control by means of reflex

arcs mediated through the sacral portion of spinal cord. The efferent pathway for these reflexes is by way of the parasympathetic fibers. The sympathetic nerves innervate the bladder from above the level of the lesion.

Stewart (1899) has demonstrated that stimulation of the parasympathetic fibers to the bladder does not give rise to a tonic contraction of the vesical muscle that continues as long as the stimulus is applied. Instead, stimulation produces rhythmical contraction waves, sometimes augmenting and sometimes decreasing previous activity of the vesical muscle.

Similarly, in our experiments, stimuli applied to the portion of the body innervated by the isolated portion of the spinal cord at times enhanced and at times diminished the activity of the vesical musculature. Stimulation of the tail or the skin and deep tissues of the perineum in almost all cases caused a contraction of the bladder. The contraction was slight following stimulation of the tip of the tail, and increased progressively as the stimulus was carried toward the perineum. Stimulation of the area of the toes supplied by the first sacral segment had almost no influence upon vesical activity. Reflex stimuli appear to travel in a cephalic direction in the cord from the coccygeal and lower sacral segments to influence vesical muscle through the parasympathetic innervation. The most effective stimuli are those which enter the second, third, and fourth sacral segments of the cord. This region gives rise to the parasympathetic fibers controlling the bladder.

Strong influence upon vesical activity was produced by stretching the anus or vagina. In general, if the intravesical pressure was low, this stimulation produced a large contraction of the vesical muscle. On the other hand, if the intravesical pressure was high or waves of contraction were present, the pressure fell and the waves stopped. Thus under certain conditions, stimulation of the anus and vagina will either increase or decrease vesical activity. These findings are similar to those reported by Stewart on stimulating the parasympathetic nerve fibers.

Denny-Brown and Robertson (1933), in their study of patients, found that stimulation of the perineum and sacral areas after transection of the spinal cord gave rise to two types of results. Stimulation

near the glans penis was likely to produce a contraction of the vesical musculature. Stimulation of areas further removed from the urethra often gave rise to contraction of the external sphincter followed by a decrease of vesical pressure. The contraction of the muscle of the external sphincter was associated with contraction of other striated muscle of the body. The authors were thus able to produce a decrease or increase of vesical contraction by peripheral stimulation. In the experimental work described here, the activity of the vesical sphincters was not studied. Our experiments can, therefore, describe only the activity of the vesical muscle.

We have previously reported the effects of stimulation and section of the sympathetic fibers to the bladder (Langworthy, Reeves and Tauber, 1934). On stimulation of the sympathetics there is an initial quick rise of vesical pressure followed by a slow fall to below the former pressure. If the pressure is held constant, there is an initial decrease of volume followed by an increase. The effect of stimulation of the sympathetics was more marked when the parasympathetic fibers to the bladder had previously been cut.

In the present experiments the sympathetic innervation of the bladder was normal. The parasympathetic innervation was controlled through the isolated portion of the spinal cord. Under these conditions the bladder muscle appeared to be unusually sensitive to stimulation through the sympathetics. Any painful stimulation applied over the portion of the body innervated above the section of the cord would in favorable conditions produce a fall of vesical pressure, and stop the rhythmical waves of vesical contraction. The same results could be obtained directly on stimulation of the sympathetic trunks, but the effect was lost after the sympathetic trunks were removed.

There are other instances in which pain stimulates the sympathetic portion of the autonomic nervous system. Thus, pinching the skin of the neck causes a reflex dilatation of the pupil. Experiments show that this reflex pathway involves the region of the hypothalamus.

In considering the control of vesical activity by the spinal cord, it is clear that the only peripheral stimuli causing vesical contraction in the cat arise from the portions of the body supplied by the sacral and coccygeal segments. The first sacral segment has little, if any,

part in this effect. The second sacral segment is the most cephalic portion of the cord to send parasympathetic fibers to the bladder. The locus of sensory area is in the region of the vagina and anus. The response becomes progressively less marked in stimulating toward the tip of the tail.

Denny-Brown and Robertson believe that the function of micturition can be carried on well by the isolated spinal cord. The bladder can be emptied completely so that no residuum of urine remains. They feel that the cerebral cortex has solely a controlling function expressed as inhibition of vesical activity. That the bladder is not usually emptied completely after spinal transection they attribute to afferent stimuli from the periphery which cause a reflex closure of the external sphincter. For our part, we assume a greater control of the bladder by the brain. We feel that this encephalic control tends to reinforce, strengthen and prolong vesical contraction, which then continues until the bladder is emptied completely. The vesical contractions observed in this study were of too short duration to empty the bladder effectively.

SUMMARY

After transection of the spinal cord in the lower lumbar region, the activity of the vesical musculature can be influenced by stimuli applied to the tail or perineal region. The contraction waves show a tendency to summation not observed after section of the sacral nerve roots. Stimuli applied to the region of the vagina or anus will produce either contraction waves or under other circumstances will cause them to cease and the intravesical pressure to fall. Stimulation of the tail gives rise to vesical contractions; the results are progressively more marked as the point of stimulation is carried centrally. Stimulation of the area supplied by the first sacral segment has no effect upon vesical activity.

Under favorable circumstances nocuous stimuli applied to any portion of the body innervated above the cord section produce a fall of intravesical pressure and stop the contraction waves. The same effect is elicited by stimulating the sympathetic fibers innervating the bladder, and is lost after the sympathetic trunks are cut.

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BOOK REVIEWS

Williams' Obstetrics. A Textbook for the Use of Students and Practitioners. By H. J. STANDER, M.D. 7th Edition. 1269 pp. Illus. $10.00. (*D. Appleton-Century Co., New York, 1936.*)

Following the death of Dr. Williams in 1931 certain qualms were expressed from time to time about the future of his textbook. It was feared that such a work, so rich in the personality of its author and so steeped in the scholarship of nineteenth century Germany, would not long survive at another's hands. Those who have entertained such fears may dismiss them. The new edition of this book, by Dr. Henricus J. Stander, not only incorporates all accepted advances, but maintains the scholarly and judicious attitude so characteristic of former editions. Several notable improvements have been made, particularly in the illustrations, and it seems probable that the book will remain the standard work on obstetrics in the English language.

The general plan of the book remains unchanged and throughout an attempt has been made to keep as much of Dr. Williams' last revision as is consistent with new developments in obstetrics. Chief among the changes is the addition of three new chapters: one dealing with the hormones as related to pregnancy and the puerperium; one discussing urinary complications, particularly pyelitis; and a third reviewing modern methods of anaesthesia and analgesia. The chapter on the sex hormones is a clear and succinct résumé of the endocrines as they relate to menstruation, pregnancy, parturition and lactation; in keeping with the tenor of the rest of the book the development of our knowledge in this field is traced from the time of Von Baer; three excellent diagrams illustrate the more important functions of the sex hormones and should prove helpful to the student. In the chapter on amnesia, analgesia and anaesthesia, most of the methods commonly used are described in detail, dosages given and contra-indications properly emphasized. In summarizing, Dr. Stander describes the practice at the New York Lying-In Hospital where he is obstetrician-in-chief. Barbituric acid derivatives with scopolamine are employed in a relatively small, carefully selected, group of patients who are cooperative and need alleviation of pain during the very early period of labor. More generally morphine and scopolamine are used, these drugs being supplemented frequently by an ether-oil mixture by rectum. For the actual delivery nitrous-oxide oxygen is administered. For home confinement, chloroform, used only as an analgesic in the second stage, is recommended. The new chapter on diseases of the kidneys and urinary tract is a welcome and distinctive contribution to the book. Illustrated by several beautiful pyelograms, this section describes in the detail they deserve the physiological and anatomical changes in the urinary tract associated with pregnancy. The importance of these alterations as etiological factors in the pyelitis of pregnancy is stressed and a

conservative program of treatment outlined. A bibliography of one hundred and eighty titles is appended to this chapter.

Among other noteworthy alterations the section on the bacteriology of puerperal infection has been entirely re-written and much new and original material introduced. The treatment of asphyxia neonatorum is also the subject of several new pages, the Flagg apparatus being strongly recommended for this emergency. In the chapter on the toxemias of pregnancy the main clinical features of these disorders are differentiated graphically in three new charts which add considerable clarity to the presentation. The new table showing in detail the diet used by the author in the treatment of hyperemesis should prove a valuable practical guide to the practitioner.

It would be strange if the reviewer, in the course of these twelve hundred pages, could not find a few points with which to take issue. The most vulnerable paragraph in the book is that dealing with the treatment of placenta previa in which occurs the following sentence: "For these reasons, we believe that the skillful obstetrician will rarely resort to Cesarean section except under two conditions —first, in the rare instances in which the cervix is too rigid to permit the introduction of a balloon, and second, in cases of complete placenta previa in women nearing the end of the child-bearing period, who are especially desirous of a living child." This limits the use of Cesarean section in placenta previa almost to the vanishing point. It is quite true that cases of marginal placenta previa as well as certain instances of partial placenta previa in multiparae can be handled satisfactorily by the vaginal route, and the routine treatment of such cases by Cesarean section is to be deprecated. On the other hand, there is growing evidence to attest that most cases of central placenta and many instances of partial placenta are most safely handled by Cesarean section. Certainly, in view of current experience, such a view as quoted above needs strong statistical evidence to render it convincing.

The format of the book has undergone several changes. The familiar maroon cover is now blue. The type and paper have been altered with the result that the pages are somewhat more pleasing to the eye than the old. The index, which was avowedly faulty in previous editions, has been reorganized and improved. Most note-worthy among the changes in make-up, however, are the beautiful illustrations by Miss Elizabeth Brödel. Not only has she contributed many new drawings but has touched up most of the old. Her work constitutes one of the most attractive and distinctive features of the new edition.

N. I. E.

A Dissertation on the Sensible and Irritable Parts of Animals. By ALBRECHT VON HALLER. (*London, J. Nourse, 1755.*) Introduction by Owsei Temkin. 49 pp. $1.00. (*Johns Hopkins Press, Baltimore, 1936.*)

The book is a reprint, with some omissions, of an anonymous English translation of Haller's classical treatise: *De partibus corporis humani sensibilibus et irritabilibus*, 1753. The original English version was published in London in 1755. Professor Temkin in his introduction gives the appropriate historical setting that

is needed by the modern reader. He rightly emphasizes the fact that the great merit of Haller's treatment of the subject is that he bases his conclusions solely upon experimental evidence. As Haller himself says "The great source of error in physic has been owing to physicians, at least a great part of them, making few or no experiments, and substituting analogy instead of them." To Haller irritability meant contractility. "I call that part of the human body irritable which becomes shorter upon being touched," and those parts are sensible which upon irritation arouse conscious sensation in man, or, in the lower animals, signs of pain and disquiet. On this basis he tested experimentally the various tissues and organs. The part was laid bare in the living animal and touched, cut, burned or lacerated, making use of the scalpel, heat, spirits of wine, oil of vitriol, butter of antimony, etc. His results as regards sensitivity to pain of the tendons, ligaments, membranes and viscera of the body were surprising to him, and indeed brought up questions which have never been fully answered. He could fill the pelvic articulation of the femur with oil of vitriol without arousing symptoms of pain. The tendons, the periosteum, the dura mater, the peritoneum could be torn, pricked or burnt without causing the least sign of pain. In modern physiology pain sensibility has been studied mainly in the skin areas. For the visceral organs and membranes our knowledge is none too satisfactory. A distinction is made usually between normal and pathological (inflamed) conditions, and Head's theory of reflected sensations gives a useful explanation of many visceral pains, but the fact remains that the path opened by Haller's experiments has not been fully explored. The great contribution attributed to Haller is that he furnished the proof of the independent irritability, or, as we would say, the independent contractility of muscle. "I shall demonstrate that irritability does not depend upon the nerves, but on the original fabric of the parts which are susceptible of it." Haller presents his conclusions and his numerous experimental observations in a clear and forceful manner. One gets a vivid picture of the controversies of the times and the difficulties that had to be overcome in establishing the basal facts of physiology. The book presents a very attractive appearance and should be welcomed by all physiologists interested in the historical development of their subject.

W. H. H.

Research in Dementia Praecox. By NOLAN D. C. LEWIS. 320 pp. $1.50. (*National Committee for Mental Hygiene, New York, 1936.*)

This volume is the first to appear on the nation-wide program for research in dementia praecox founded by the Northern Masonic Jurisdiction of the Scottish Rite and under the general direction of the author, recently Associate Professor of Neurology at the New York Neurological Institute, and now Professor of Psychiatry at Columbia University and Director of the New York Psychiatric Institute. The writer has made a comprehensive attempt to cover the literature of the last fifteen years in every phase of research on schizophrenia. The chapters indicate the scope—Research Trends in Mental Disorder, followed by excellent

summaries and accurate bibliographies in the Clinical Features, Etiological Aspects, Alterations in Structure and Structure Function, Differential Diagnosis, Therapeutic Modifications and Experimental Therapy, and finally with the Conclusions. In keeping with the author's own special interests, the material on Alterations in Structure and Structure Function is more extensive than in the other categories. In addition to the thorough search of literature of the past fifteen years, there is a bibliography of books dealing with dementia praecox and also of important literature bearing on the subject which appeared before 1920.

The book is one which no one interested in the problem of dementia praecox and acquainted with its magnitude and difficulties can afford to be without.

W. M.

Principles of Human Physiology. By ERNEST H. STARLING. 7th ed. Edited and revised by C. Lovatt Evans and H. Hartridge. 1096 pp. Illus. $8.75. (*Lea and Febiger, Philadelphia, 1936.*)

This book has many desirable features, among them being the introduction in which physiology is defined and the general aims with the methods and their limitations are discussed. The references given at the end of each chapter are very valuable for the student wishing for more information. The placing in fine print of the description of methods and the discussion of controversial material is to be praised. The chief fault of the book consists in the large amount of space (106 pages) devoted to a general consideration of osmotic pressure, colloidal chemistry, types of chemical changes and other biochemical subjects inasmuch as these are treated much better and in greater detail in textbooks of physiological chemistry.

In this edition there has been sufficient revision to warrant a continued recommendation of the text for student use in medical schools.

C. L. G.

Squint Training. By M. A. PUGH, M.R.C.S. (Eng.), L.R.C.P. (London). 117 pp. Illus. $2.75. (*Oxford University Press, New York & London, 1936.*)

This is a small volume of 111 pages on the classification of ocular strabismus, orthoptic training, and the correction of squint by various means. It is divided into eight short chapters, with an appendix which is little more than a description of the various kinds of commercial apparatus used in orthoptic training. No attempt is made to deal with the academic side of the question. The value of the book lies, first, in the definite need for such a compend; second, in the clear, concise manner in which the subjects are set forth, and the evident honesty of the statistics presented.

While there is still some skepticism expressed on the actual value of orthoptic training, there is a growing conviction among ophthalmologists that it should play an essential part in the correction of a large percentage of cases of strabismus. There is as yet no unanimity of opinion as to the most valuable of the numerous methods available, and the whole subject is somewhat obscured by the extravagant

claims of the manufacturers for their different devices. In this practical manual the author has kept well to the middle of the road, and has simply outlined the methods and recommended the apparatus she has found most valuable. Advocates of other methods will take exception to some of the recommendations, but in the light of our present knowledge they appear sound and the volume will be of value to practical workers and technicians in this field.

A. C. W.

Encyclopedia of Sexual Knowledge. By A. COSTLER, A. WILLY and others. Edited by Norman Haire. 567 pp. Regular price $6.00. Popular priced edition $3.00. (*Eugenics Publishing Co., New York, 1934.*)

This work originally compiled by several French sexologists and now translated and edited by Norman Haire, an English authority on sex and birth control, might better be entitled "A Résumé of our Sexual Knowledge." Almost invariably the author of each section cuts his discussion short, alleging lack of space.

Instead of starting a book of this kind with the usual description of the sex organs the authors have preceded this portion with an introductory chapter sketching the evolution of love.

The first part of the book is devoted to tracing the development of sex life. The chapters dealing with masturbation, discounting the older theories of the untold harm this practice exerts upon the human body, are particularly good. On the other hand, the section on the relationship of the glands of internal secretion is some years out of date. The discussion of menstrual hygiene certainly does not conform to the ideas of modern gynecologists.

The second division of the book deals with sexual intercourse and, while somewhat brief, is quite good. Following this is a sketchy and elementary chapter on procreation.

The fourth section takes up the imperfections of love. In this are included discussions on sterility, impotence and a very enlightening chapter on the alchemy of love. Sadly neglected in this section is the chapter on contraception owing, as the publishers explain, to our prohibitive laws. The original chapter has apparently been omitted and a very inadequate one substituted. The section ends with a chapter on "rejuvenation," the subject matter of which probably will not meet the approval of most modern students of the subject.

The fifth division consists of a discussion of sexual aberrations. This is quite full and unusually instructive.

The sixth and last section deals with venereal disease. One wonders, while reading it, whether it could have been written by a physician; surely by neither a gynecologist nor a syphilologist.

Taken all in all the book is well worthwhile. Not a finished piece of work by any means, it serves as a contribution to a very important branch of medical science which has never been brought before the medical profession or the public with proper emphasis.

J. H. L.

Diseases of the Coronary Arteries and Cardiac Pain. Edited by ROBERT L. LEVY. 445 pp. Illus. $6.00. (*The Macmillan Co., New York, 1936.*)

The last 25 years have witnessed encouraging advances in the clinical description of diseases of the coronary arteries, particularly in the recognition of coronary thrombosis. Formerly understood principally in terms of the results observed *post mortem*, this condition has been proved susceptible of diagnosis during life and has been studied and described in detail.

Now appears this volume, which, in that it makes available to all physicians an authoritative summary of the knowledge of its domain, should deserve rank among the notable contributions to the understanding of these diseases. It is written by fourteen physicians, each abundantly qualified by experience and by previous contributions to the subject. After a challenging introduction by Dr. Alfred E. Cohn and a short view in retrospect by Dr. James B. Herrick it presents the biological foundations of the diseases concerned, their characteristic manifestations in terms of morbid anatomy and physiology and the influence of therapeutic measures including surgery. It is remarkably coherent with a minimum of repetition.

The contributors are by no means in agreement among themselves upon all details but such divergence of opinion serves only to emphasize that, on these points as on others, knowledge may not be complete. Such incompleteness is acknowledged in many instances, for example, in the discussion of the dynamics and nervous control of the coronary circulation. Elsewhere it is implied, and nowhere more strikingly than in the use of the terms "angina pectoris" and "cardiac pain." By some of the contributors the former term is applied only to the pain apparently due to myocardial anoxemia consequent upon disease of the coronary arteries, and not to the pain associated with coronary occlusion. By others the term is diffused to cover less specific conditions with or without definite anatomical implications.

The editor and the contributors are to be congratulated upon the preparation of the text and the bibliography. The book is to be recommended highly to all medical students and physicians.

E. C. A.

Skin Diseases in Children. By GEORGE M. MACKEE, M.D., and ANTHONY CIPOLLARO, M.D. 345 pp. Illus. $5.50. (*Paul B. Hoeber, Inc., New York, 1936.*)

This is a comparatively small book of 333 pages and 153 illustrations which the authors have attempted to simplify as regards classification and description. The chapters on treatment have been handled in a concise but efficient manner and are not overburdened by the enumeration of a great number of special methods of unproved value which fill up so much room in many of the larger texts. The photographs as a whole are good but some of them could be improved.

It can be recommended to the pediatrician and also to the general physician who are looking for a book emphasizing cutaneous medicine in children.

L. K.

Plastic Surgery of the Nose. By J. EASTMAN SHEEHAN. 2nd edit. 186 pp. Illus. $9.00 (*Hoeber's Surgical Monographs, Paul B. Hoeber, Inc., New York, 1936.*)

A decided improvement over the first edition published in 1925, both in text and illustrations.

The classification of nasal deformities in a general way follows that of J. Joseph and naturally the schematic drawings are similar. A few typical operations for nasal deformities are given in detail. Attention is called to the magnificent and highly technical work of Mr. E. A. Hardy who uses prosthetic appliances in nasal and premaxillary reconstruction. Free skin grafts and flaps are discussed broadly, with illustrations of the author's methods. A theory for keloid formation and another method of treating these tumors are included.

The book is too short to give more than a very superficial review of plastic surgery of the nose and is mainly interesting in that it expresses the views and methods of one surgeon.

E. A. K.